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MEASUREMENTS OF IONIZATION IN THE KENNELLY-HEAVISIDE LAYER DURING THE SOLAR ECLIPSE OF 1932¹

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Abstract

The ionization of the Kennelly-Heaviside layer was measured by Appleton's method during the total solar eclipse of August 31, 1932. In contrast to normal days, a sudden decrease of 58% during the period of optical totality was found. This is shown to be in accord with Appleton's, but not with Chapman's, theory that the cause of lower layer ionization is ultra-violet light from the sun. Some measurements were made of the effective height of the Appleton layer and these are discussed in detail. There is a marked effect at the time of optical totality on this layer also.

Introduction

In the early days of radiotelegraphy great difficulty was experienced in explaining how electromagnetic waves travel so far round the earth. At about the same time Kennelly in the United States, and Heaviside in England suggested that there might be an electrically conducting layer in the atmosphere which reflected, or bent, the radio waves down towards the earth. It was not until 1925 that Appleton and Barnett (4) proved the existence of such a layer by direct experiment, and in 1926 the point was confirmed by Breit and Tuve (7) using a different method. About a year later Appleton demonstrated (2, 3)* that there were in reality two such regions, the lower one called the Kennelly-Heaviside layer, or E region, being some 100 km. above the earth's surface, and the upper one, the Appleton layer, or F region, being about 250 km. high.

The cause of the existence of these layers was naturally the subject of speculation, and the special advantages of investigating the electrical state of the upper atmosphere by radio methods during a total solar eclipse have been emphasized recently by Appleton (6) and by Chapman (8). They have pointed out that such observations would discriminate between the rival theories concerning the origin of ionization in the lower conducting layer.

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* See also Reference (12).

Advantage was therefore taken of the total solar eclipse of August 31, 1932 by many investigators, to test some of the points at issue. The results obtained by the Canadian expeditions are described in this and the accompanying papers (9, 11).

During the total eclipse of 1927, Appleton made observations in which the relative signal strength of the downcoming wave was compared with the strength of the ground wave, and the ratio was found to increase at the time of totality. The height of the Kennelly-Heaviside layer, measured by the frequency change method, was also found to increase at the time of the eclipse. The observations have since been interpreted by Appleton as being strong evidence in favor of the hypothesis that ultra-violet light from the sun was a principal agent in the production of ions in the lower layer. Professor Chapman, however, was inclined to attribute the cause of ionization to neutral particles shot out from the sun by radiation pressure. The difference to be expected in the effect of an eclipse, if the cause of lower layer ionization be particles on the one hand, or ultra-violet light on the other, has been discussed by Chapman (8),* and by Appleton and Chapman (5).

The map reproduced on page 35 gives the positions of the central line of the umbral shadow on the ground, both at a height of 100 km. and at a height of 200 km. It also shows the southern limit of the expected corpuscular, or particle, eclipse. The "shadow" extends eastward as far as Europe, the central line running through Greenland and the Atlantic Ocean. This "corpuscular" shadow is displaced from the optical shadow in place and in time. If therefore the ultra-violet theory is correct the maximum reduction of ionization due to the removal of the producing agent should take place about the time of optical totality, and the best position for observing it would be under the shadow on the lower layer. If the corpuscular theory is correct, then the maximum reduction in ionization should take place some two hours earlier and the best position for observing it would be over the Atlantic Ocean.

To satisfy these conditions as nearly as possible, one party was located at Vankleek Hill, Ontario, directly below the shadow at 100 km., and one party at Corner Brook, Newfoundland, about 300 km. east of the predicted western boundary of the corpuscular eclipse.

The method adopted in the present investigation was that, developed by Professor Appleton (6), of directly measuring the ionic content of the layer, the object being to follow the changes in ionic density in the lower layer throughout the eclipse period.

Experimental Details

The procedure was to use the well-known impulse method of Breit and Tuve (7). Very short signals are sent out from a transmitting station and reach the receiver by two distinct paths, one ray travelling along the surface of the ground, and one travelling up to the reflecting region and back to the

* See also Reference (10).

receiver. The retardation of the second signal behind the first, or direct wave, is a measure of the equivalent height to which the indirect, or sky wave, has travelled. The experiment is further simplified by placing the sending and receiving stations as close together as is convenient (*e.g.*, 2.7 km. at Vankleek Hill), so that reflection takes place at normal incidence.

At the receiving station, the signal, after suitable amplification, is rectified and impressed on a cathode ray oscillograph. Visual observation and photographic recording are maintained simultaneously.

To measure the ionic content, the frequency of the transmitter is increased step by step. At first the reflected wave or echo is found to come from the lower layer (Kennelly-Heaviside, or E, region). As the frequency increases, for all frequencies greater than a certain critical value the echo no longer returns from region E but comes from the upper, or F, region (Appleton layer). This critical penetration frequency gives a count of the maximum number of ionic carriers present in the layer. The relation here used to calculate the ionic density is $f^2 = kN$, where f is the penetration frequency observed, $k = 0.184 \times 10^{-7}$, and N is the number of electrons per cubic centimetre in the Heaviside layer. The assumptions involved and the limitations of the above formula are discussed by Appleton and Naismith (6).

Apparatus

Transmitting Stations

The transmitter used is a simple tuned-plate tuned-grid circuit. To emit the short signals necessary the transmitter is keyed in the common negative lead by a mechanical commutator driven by a synchronous motor. The length of the impulses, using this form of keying, varied from 0.3 to 0.5 millisecon. (measured on the receiver oscillograph). Although longer than desired, they are not so long as to inhibit accurate measurement of the critical penetration frequency. The recurrence frequency of the impulses is 30 per sec.

The aerial is fed directly through a tap on the anode coil. At Vankleek Hill the aerial system is a simple inverted L, mounted on masts 60 ft. high, with a horizontal top about 250 ft. long. The dimensions of the Corner Brook aerial are only slightly smaller.

The experiment is, of course, controlled from the receiving end which is in telephonic communication with the sending station. The wave-length is, however, measured at the transmitter, a General Radio precision wavemeter being used as a reference.

Receiving Stations

The receiver for Vankleek Hill was constructed at the Radio Research Station, Slough, and follows the current English design of receivers for this type of work. It is a superheterodyne set with one tuned stage of signal frequency amplification. The output of the local oscillator can be adjusted

for optimum heterodyne. The I.F. amplifier has a gain of 90 db. and a flat band pass of 15 Kc. (kilocycles per sec.) centered on $112\frac{1}{2}$ Kc., the cut-off being fairly sharp on both sides of the band.

The output to the oscillograph is taken directly from the second detector to the plates of the oscillograph, audio-frequency amplification being unnecessary and not very desirable. By using a diode rectifier the first 0–20 volts output is made linear with input. From 20 to the maximum output of 65 volts this linearity is departed from, but that is not important, since the point of making the over-all characteristic linear is to ensure that the small echoes (*i.e.*, weak signals) show on the oscillograph screen.

The Corner Brook receiver was a recent commercial model. The only alteration made was to cut out an audio-frequency stage fed through transformers, as this form of coupling is not suitable for echo work. One resistance-coupled audio-frequency stage was left in, the output of the oscillograph being taken across a resistance of 5000 ohms.

A linear time base is supplied, and to calibrate it the note from a 1000-cycle hummer (General Radio) is put on the oscillograph (replacing the echo pattern) after each change of transmitter wave-length and photographed. The time spread used varied throughout the experiment according to the number of multiple echoes it was desired to photograph, but a common spread is 1.5 cm. per millisecond, so that time intervals can be measured fairly accurately.

Much of the preparatory work was done in England at the Radio Research Station, and at King's College. The apparatus was then installed in McGill University, Montreal, for preliminary tests and observations. The heights of the ionized layers were first measured on July 19. The equipment was later moved to Vankleek Hill and Corner Brook, the experiments proper being done between August 28 and September 3, inclusive. The time of observation was from 1500 to 2400 G.M.T.*

Results

A. Vankleek Hill, Ontario

Every half-hour photographs were taken on the three fixed frequencies, 4.0, 3.16, 2.73, Mc.† (75, 95, 110 m.). The first of these values was chosen to give upper layer reflection always, the second is the critical penetration frequency for 1830 G.M.T., and the third is the critical penetration frequency for 2030 G.M.T., both for normal days. The remainder of each half-hour was devoted to determining the penetration frequency for that particular period, which was found as often as possible. Generally speaking the accuracy of the determination is good (usually about 2 or 3% and sometimes as high as $\frac{1}{2}$ of 1%), but when echoes from both layers are found over a wide band of frequencies, or when the echoes become very small, and sometimes disappear entirely over a small range of frequencies close to the critical one, then the accuracy is naturally diminished in proportion.

* In this notation 1918 G.M.T., for example, means 19 hr. 18 min. Greenwich Mean Time.

† Mc. means megacycles per sec.

One further difficulty was encountered on August 28, 29 and 30. For each of these days there appears one blank interval during which it was impossible to get echoes on any wave-length at our command (*viz.*, 45-170 m.). These periods are:

August 28.....1610 to 1918 G.M.T. (3 hr. 8 min.)

August 29.....1950 to 2210 G.M.T. (2 hr. 20 min.)

August 30.....2118 to 2310 G.M.T. (1 hr. 52 min.)

which decrease in length and appear later in the day. No such blank periods were noticed after August 30; nor were they noticed on the previous week during the preliminary tests.

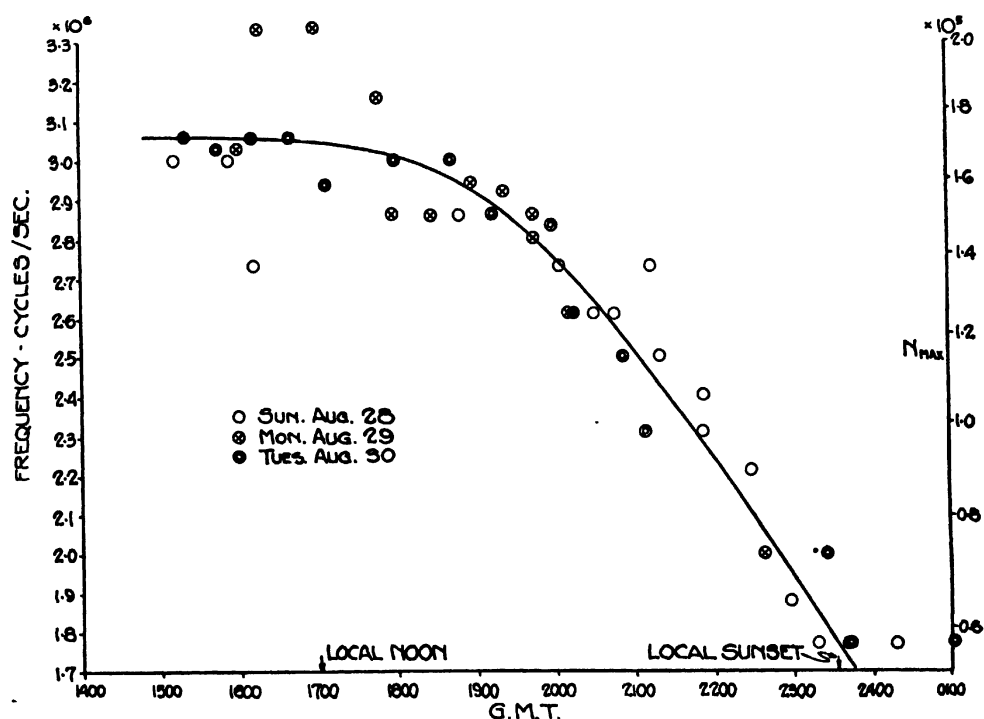


FIG. 1. Critical frequency on the left and ion density on the right plotted against time, for the three successive days preceding the eclipse.

On September 1, the recording was interrupted from 1618 to 2302 G.M.T. by a local thunderstorm of considerable violence. Despite these blank intervals it is felt that the curve presented in Fig. 1, showing the variation of ionic density in the Heaviside layer throughout the day, is a good approximation to the actual conditions existing on a normal day at this time of year. The points are shown for August 28, 29, 30, only, but those for September 2 and 3 fit equally well on this same curve, and have been omitted only to avoid confusion.

In Fig. 2 is shown the same curve for August 31, 1932. In this graph the inked-in circles represent frequencies showing lower layer reflection. The open circles are frequencies reflected from the upper layer. A circle half black denotes either the actual critical frequency, or a frequency showing reflection from both regions. (When it is not possible to give the exact value, the critical frequency will obviously lie between a point which shows reflection from the lower layer and one which shows reflection from the upper layer.) There is no doubt about the coincidence of the removal of the ionizing agent with the shutting off of the sun's light by the moon.

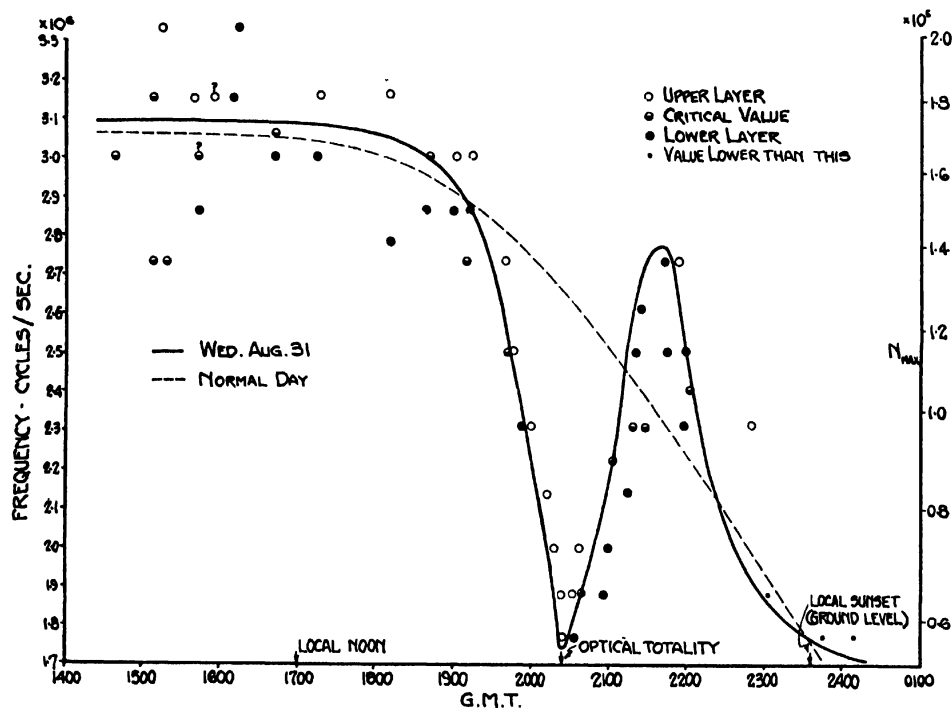


FIG. 2. Critical frequency on the left and ion density on the right plotted against time, for August 31, the day of the eclipse.

difficulty was not in noticing the effect, but in following the rapidly changing critical frequency. The penetration of the lower layer by a frequency of 1.72 Mc. (170 m.) at a time of day when 2.72 Mc. (110 m.) is only just going through, and the subsequent reflection of this abnormally low frequency by the re-forming E region ten minutes after totality is, in itself, sufficiently striking. No abnormal behavior was noticed on August 31 before about 1915 G.M.T., and certainly conditions in the layer were normal at 1830 G.M.T.

It will be noted that, after the eclipse, the ionization increases between 2100 and 2200 G.M.T. to a value higher than normal. The duration of the electrical effect is about two hours, judging solely by the width of the sudden depression in the ionization curve. The maximum effect (*i.e.*, minimum ionization) coincides with totality to within three minutes.

Fig. 3 gives the variation of the equivalent height throughout the day for 2.73 Mc. Normally this frequency is returned from the lower layer until about 2030 G.M.T., when it penetrates and is then returned from the upper layer. This condition is illustrated in the dot and dash curve, which is the curve for the other six days of the experiment, and is considered to represent normal conditions at this season. Actually the normal time of penetration is best represented by an area, as this time varies slightly.

On the day of the eclipse, conditions were much as usual until about 1930 G.M.T. when penetration occurred about an hour earlier. The height of the upper layer instead of remaining roughly constant showed a sudden increase shortly after totality, with a return to more normal heights immediately afterward. There was another slight increase in height about 2240

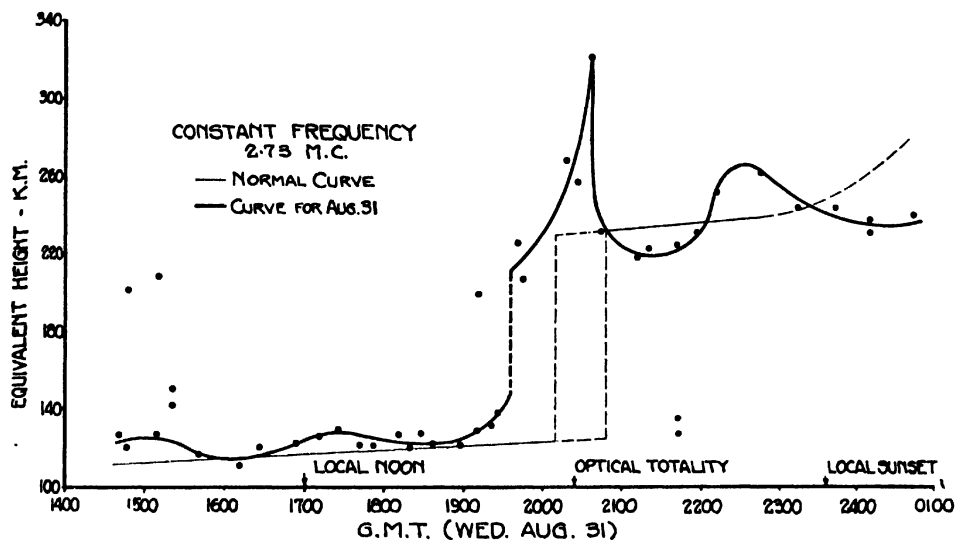


FIG. 3. Equivalent height of reflecting layer for 2.73 Mc. constant frequency, plotted against time. Contrast between normal curve and curve for August 31 indicated.

G.M.T., just a little before ground sunset (*i.e.*, 2336 G.M.T.), but as this is sometimes found on other days also, it is not considered very abnormal. The point at 2026 G.M.T. was observed for four minutes, and the height is based on 22 pictures taken at this time.

The isolated points at 2145 G.M.T. are interesting. In the first place they show that the ionization in the lower layer was strong enough to return part of the energy on this frequency, thereby indicating an abnormally high ionic content in region E. Secondly, four echoes show on 8 of the 12 photographs taken at this time, but the measured heights vary considerably in the different pictures so that we are undoubtedly dealing with a temporary and unstable state. The greatest part of the reflected energy is in the F'_1 echo*, whose

* The echoes are named for the region from which they are returned. Thus E_1, E_2, E_3, \dots are echoes from the E region, the suffixes denoting whether they are the first, second, or third reflections. Primed letters, *e.g.* F'_1, F''_1 , denote the components of the n^{th} reflection of an echo showing magneto-ionic splitting.

height is constant. The E_1 echo shows only spasmodically; F_2 shows occasionally. The photographs are reproduced in Plate IV and are discussed more fully below. The equivalent heights plotted in Figs. 3 and 4 are calculated from the time delays to the first sign of an echo, except where echoes from both layers are present as in this instance, and again between 1400 and 1500 G.M.T.

The split components are not shown separately, because the rather long impulse used does not permit our separating them with any pretence at accuracy. Multiple echoes are not marked on the curves, as the energy will be returned from the regions shown since multiple echoes are interpreted as being due to multiple reflections from the same height.

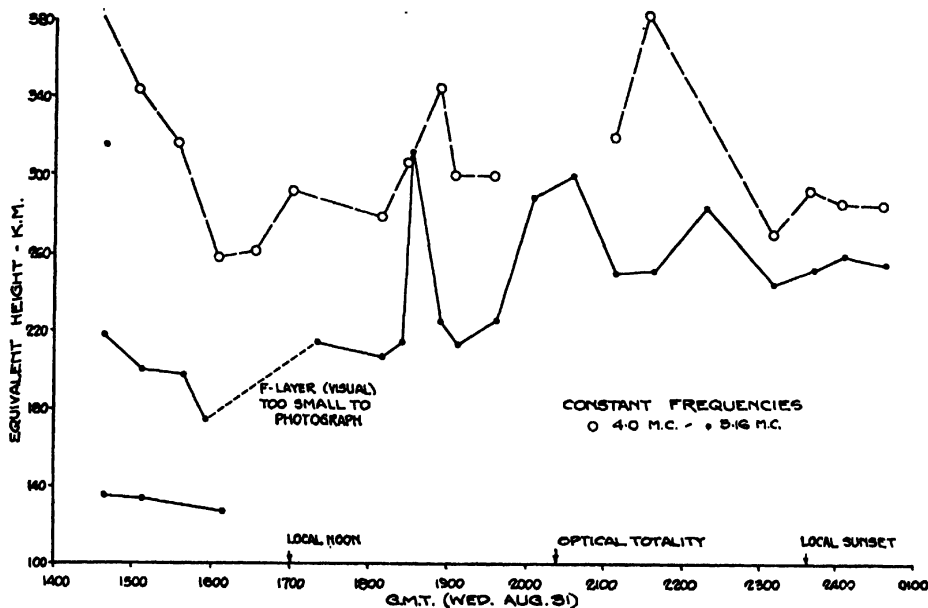


FIG. 4. Equivalent height of reflecting layer for 4.0 and 3.16 Mc. respectively, plotted against time.

The lower curve in Fig. 4 is for 3.16 Mc. Ordinarily this frequency is just on the border line and may show lower layer echoes for a few hours about midday when region E is most intensely ionized. Unfortunately the frequency is slightly high and on several days the maximum noon ionization was not sufficiently great to reflect this frequency. All the control days of the experiment show upper layer reflections after about 1830 G.M.T., and a gradual increase of height with time until about 2200 or 2300 G.M.T., when there is a slight fall.

On August 31 this frequency gives only occasional lower layer echoes in the early forenoon and upper layer from 1630 G.M.T. onwards. There seems to be a sudden increase of height at 1830 G.M.T., lasting only a short time and based on four pictures taken in three-minute observations. There is a more marked increase between 2000 and 2100 G.M.T., and a minor increase at 2230 G.M.T.

The broken line represents layer heights for 4.0 Mc. and has roughly the same general shape as that for 3.16 Mc. Unfortunately the most interesting part of the curve is missing, because about totality the noise and interference from other stations increased so much that photographic and visual observations were impossible. Judging from reception of outside stations night-time conditions were restored about totality.

It is well known that conditions in the ionized layers are extremely variable. For 4.0 Mc. this statement is only too true, and the "normal" conditions are very difficult to determine from the erratic curves obtained on the six control days. All that can be fairly said is that there is no sudden appearance of night-time reception conditions.

Of the three equivalent height curves shown, that for 2.73 Mc. has the most weight, and that for 4.0 Mc. the least, Fig. 4 being presented chiefly for comparison with Fig. 3. Of course the character of the photographs is a considerable aid in drawing the curve. Thus the point on the 2.73 Mc. curve at 2036 G.M.T. is not dismissed lightly as a single observation, because it is based on a little longer period of observation than the others and on a greater number of pictures. Conditions on all three frequencies about totality resemble night-time conditions more closely than at any other time of the day (*viz.*, larger echoes, multiples more frequent, and more noise and general interference). Although none of the reproductions show good examples of multiple splitting, these are frequently observed in the evening and at night, three echoes from the upper layer being quite common. In the daytime the general rule is one or two echoes, with the second often quite small, so that the appearance of three large echoes at totality, with signs of a fourth, on 2.73 Mc. (110 m.), was quite a spectacular result.

In Plates I to V are shown some samples of the photographic evidence from which the above curves are drawn. The photographs are a long series of individual exposures and, for purposes of reproduction, have been re-photographed on a reduced scale, the original records being 10 cm. wide. Plates I to III show three columns to be read downwards in succession (starting with the left-hand one). Plate V may be regarded as the magnified reproduction of an omitted third column in Plate IV. *The temporal sequence of events is from right to left in all cases.* The small figures under the row give the time of the observation (G.M.T.) and the wave-length in metres. With the exception of Plate V all photographs were taken on August 31.

Thus the first column (Plate I) shows three pictures of upper layer echoes on 75 m. The ground wave (the first signal) is considerably larger than the echo. The equivalent height in this case is 343 km. The time interval between successive photographs is small, and throughout the whole of the records it is often observed that the echo varies rapidly in character and amplitude. Below these three pictures are two photographs of the 1000-cycle calibration frequency. The wave form is distorted and the pattern superimposed twice on the screen, so that the distance between *alternate* peaks is one millisecond. The return stroke of the time base also shows, but has no significance here.

In column 2 are shown lower layer echoes on 2.73 Mc. (110 m.). Some noise is apparent on this frequency, but the photographs illustrate the ease with which the echo pattern can be seen through the interference, this being one of the chief advantages of the method. At 1625 G.M.T., 2.73 Mc. was reflected from the Kennelly-Heaviside layer, but between 1926 and 1940 G.M.T. this frequency had penetrated the lower layer, and at the top of column 3 will be seen the upper layer echoes taken at 1940 G.M.T.

Below this are four photographs on 2.50 Mc. (120 m.). The equivalent height is about 140 km.. The pictures show the complicated splitting that usually occurs just about the critical frequency. Five minutes later this frequency had penetrated the lower layer and was being returned from an effective height of 225 km.

Plate II, column 1, shows a large increase in the magnitude of the F layer echoes on 3.16 Mc. (95 m.) 17 min. before totality, and this presumably indicates decreased absorption in the E region. The F layer echoes on 110 m., column 2, (taken at 2008 G.M.T.) were approaching normal night-time magnitude and complexity. Small second reflections can be seen on three pictures, and the rapidly changing shape of echo is marked. In the third column is shown the penetration of the lower layer by 1.88 Mc. (160 m.) one minute before the eclipse. The noise was then much worse than normal.

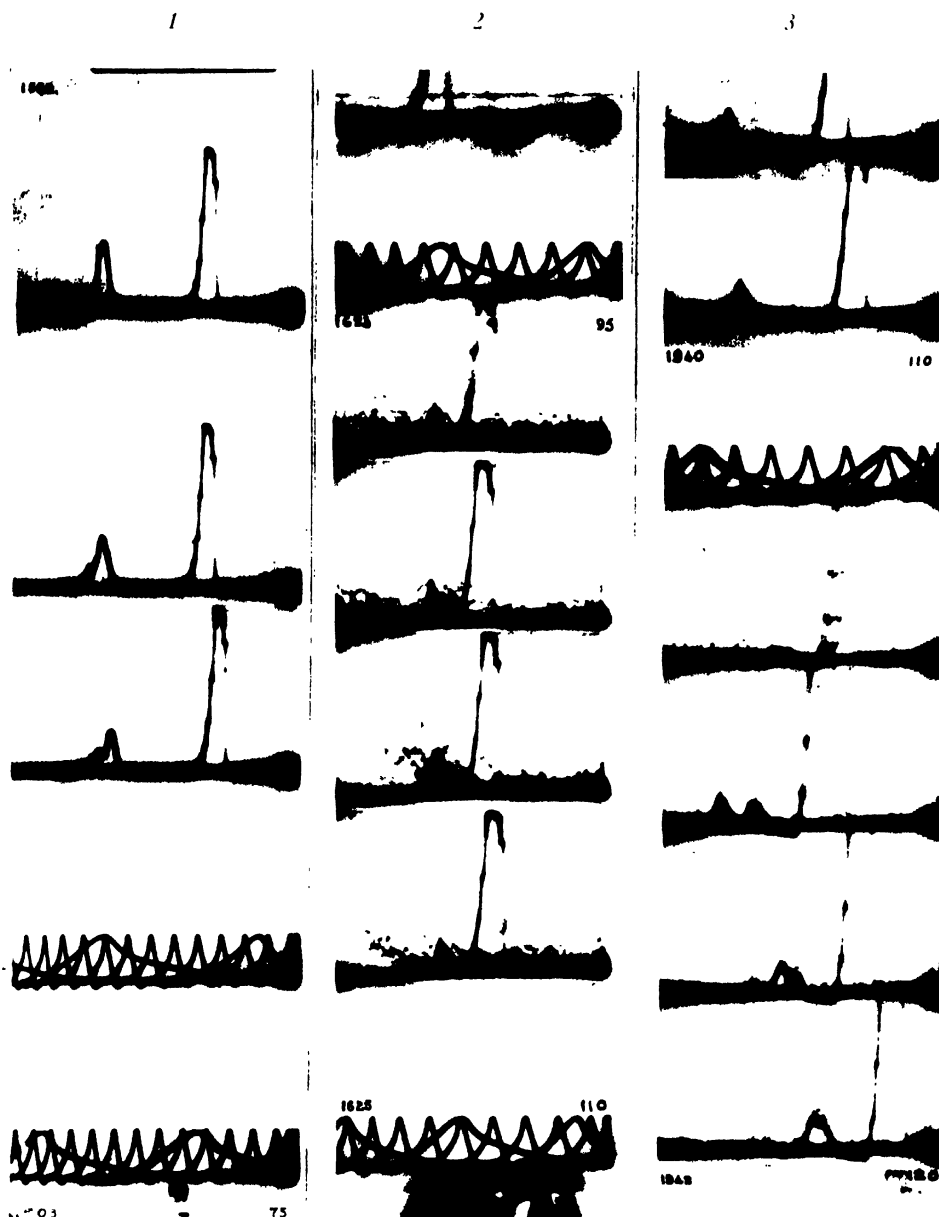
Plate III shows the penetration of the E layer at 2025 G.M.T. (one minute after totality) by 1.77 Mc. (170 m.) the height being 222 km. Ten minutes after the eclipse this wave-length is again being reflected from the E region (see bottom of second column).

In the previous plate it was seen that 160 m. was penetrating the E region at 2023 G.M.T. At 2031 G.M.T. the height was 188 km.; at 2040 G.M.T. it was 145 km., and by 2058 G.M.T. this wave-length was being returned from 122 km., which is a normal value of the height of the Heaviside layer (see columns 2 and 3).

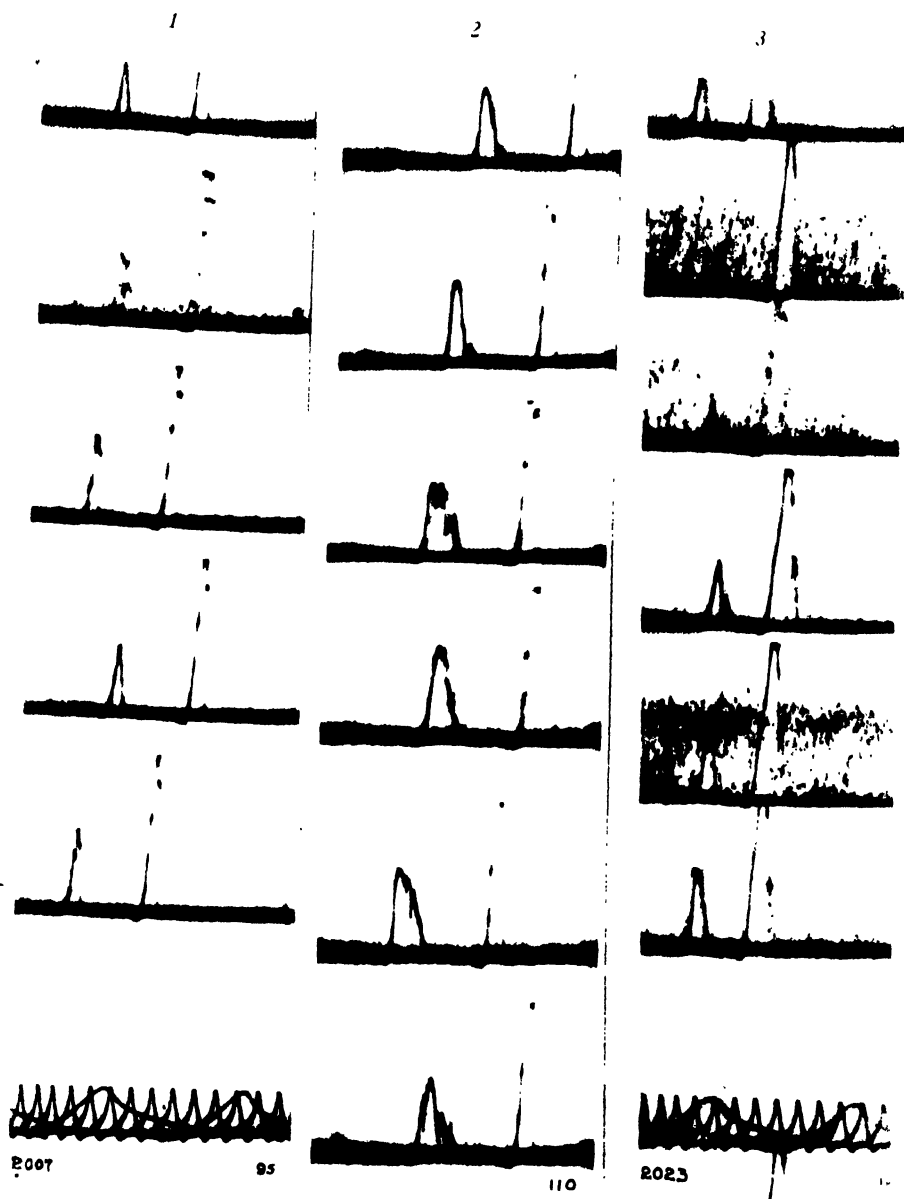
The behavior observed on the frequencies 1.77 and 1.88 Mc. is positive evidence of the abnormally low ionic content in region E.

Plate IV shows the complex echoes on August 31 at 2146 G.M.T. on 2.73 Mc. (110 m.). It will be observed that the F_1 echo is always present and by far the largest. It is the echo whose time delay corresponds to the height shown on the curve in Fig. 3. The E echo is present only on some of the pictures, so this frequency presumably gives the peak of the increasing lower layer ionization curve, after the eclipse. In row 2 of column 1, the various components are labelled. The calibration frequency for timing the echoes in column 1 is at the top of column 2. After this the time spread was changed and the last row in column 2 gives the calibration frequency for the remaining five photographs, 12 in all being taken on this frequency at the time in question.

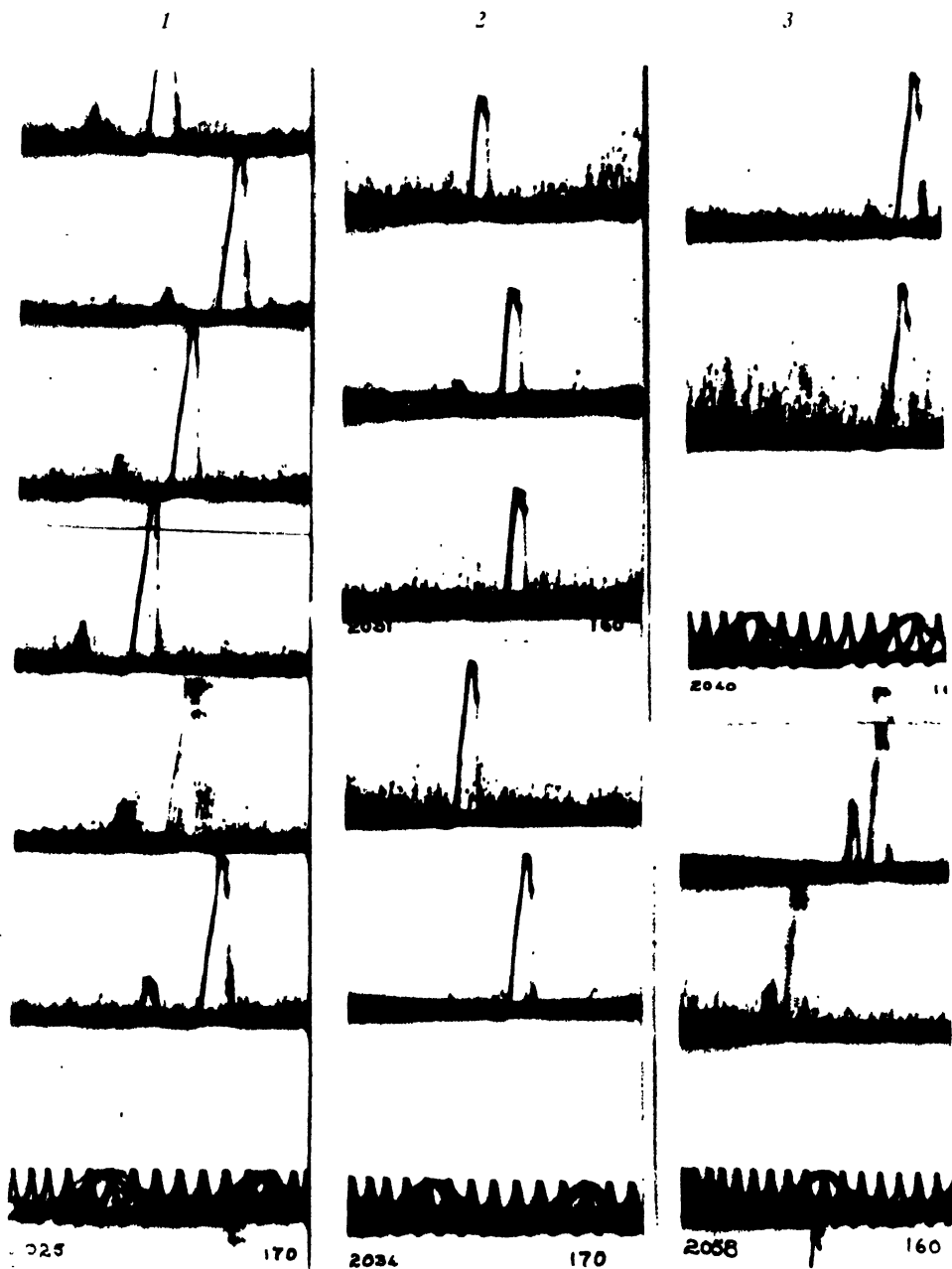
Plate V is the only set of reproductions not taken on August 31. It is on 4.0 Mc. (75 m.) at 1630 G.M.T., on September 2, and shows a good example of simultaneous reflection from both layers. Incidentally this is rather a high frequency to show a lower layer echo.



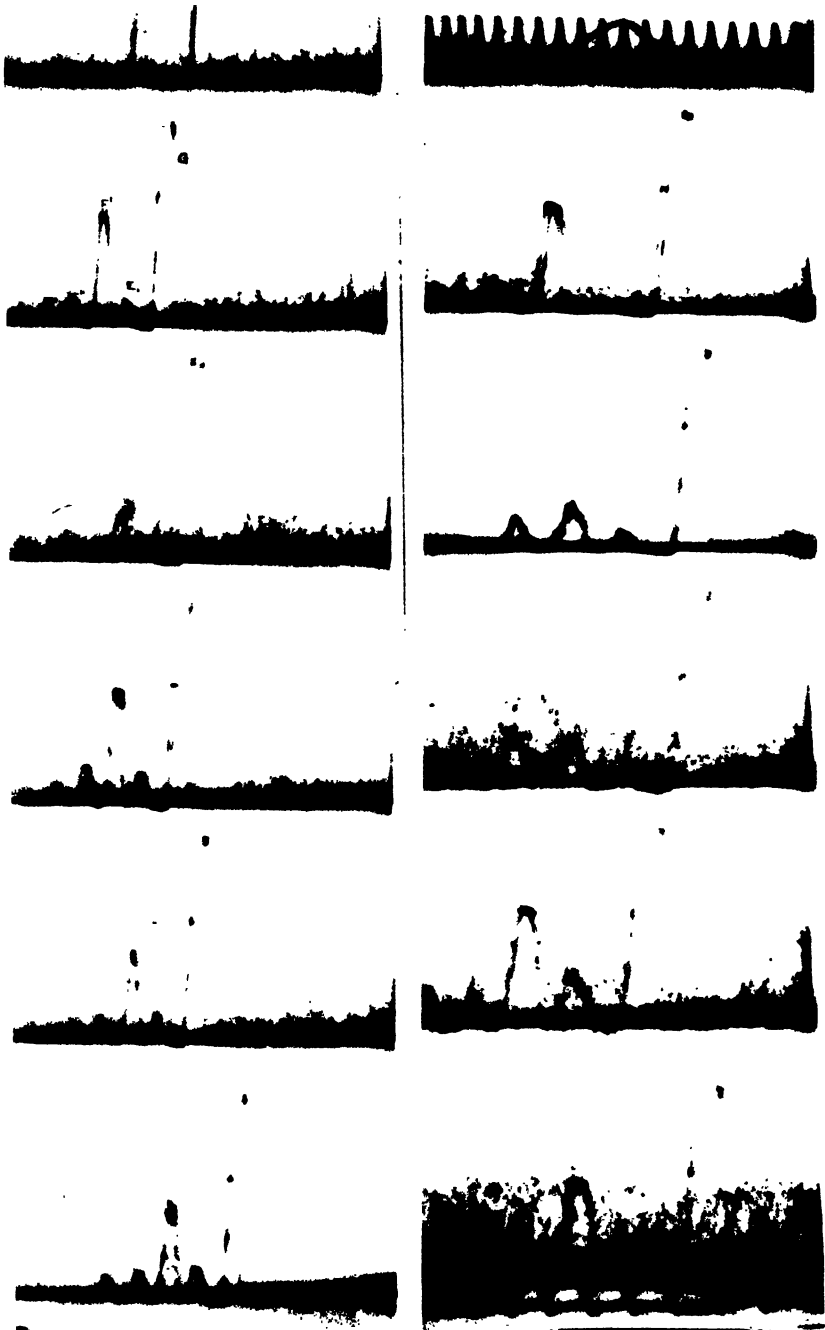
Echo patterns showing reflection from lower layer and eventual penetration to upper layer, also 1000-cycle time scale calibration. (Read patterns from right to left. Ground wave is large pulse at the right in each case).



Echo patterns on 3.16 and 1.88 Mc. just before totality. (Note the noise appearing in column 3, confirming approach of night-time conditions).

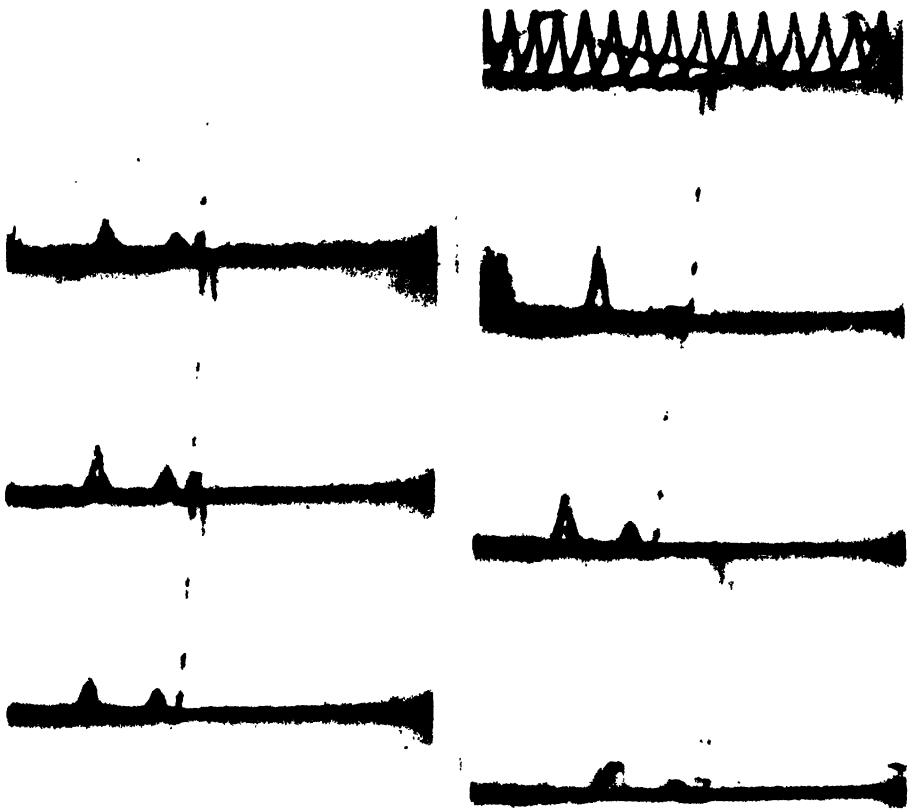


Echo patterns on 1.77 and 1.88 Mc. at and following moment of totality. (Note change from upper layer to lower layer reflection at bottom of column 2, 10 minutes after totality).



Echo patterns showing complex splitting of reflections on 2.73 Mc. following totality. (Components are labelled in second row of column 1).

2



Echo patterns showing simultaneous reflections from both layers on 1.6 Mc. (Taken on Sept. 2, at 16.30 G.M.T.).

B. Corner Brook, Newfoundland

Here the evidence presented, although of a less convincing nature, can be used in support of the main conclusions to be drawn from the Vankleek Hill observations. The expedition was hampered by lack of time and a location very difficult to work in, owing to the extremely high electrical noise level encountered and the rather unsuitable terrain. The choice of observing site was governed by the question of power supply, this forcing a selection which otherwise might have been improved.

Observations were consequently delayed until August 31, but continued until September 3. Lower layer echoes were observed on two occasions only, so that the existence of lower layer echoes was inferred from the disappearance of upper layer echoes as the frequency of the transmitter was reduced. The method is somewhat analogous to the determination of the critical penetration frequency of the Appleton layer where this is determined by noting the maximum frequency for which upper layer echoes can be obtained, but the inversion of the process is less legitimate here. Only relatively large echoes give any indication of their presence, so for the most part even upper layer echoes are lacking. The upper layer echoes, however, do show through at night fairly regularly in consequence of the decreased absorption in the lower layer permitting a greater return of energy, so that on the day of the eclipse a return to night-time conditions would be inferred if these echoes are obtained.

The experimental procedure was similar to that at Vankleek Hill. The fixed frequencies 2.7 and 3.2 Mc. were observed and photographed every half-hour. A rough attempt to find the critical penetration frequency for the lower layer, by noting the minimum frequency on which the upper layer echoes could be obtained, was made in the remaining portion of the half-hour. The critical frequency obtained in this way would tend to be too high, because often the magnitude of the upper layer echoes decreases as the critical frequency is approached. Unfortunately, intercommunication troubles between the stations rendered this determination very difficult.

On August 31, no echoes were seen until 2030 G.M.T. on 3.3 Mc. Echoes were first photographed at 2100 G.M.T. when the equivalent height was 250 km. for a frequency of 2.7 Mc.

On September 1, 2, and 3, echoes were not obtained until 2300, 2130, 2130 G.M.T. respectively (being first seen on the higher frequency and then on the lower, as would be expected). Local time is $2\frac{1}{2}$ hr. earlier than G.M.T. and ground sunset was at 1846 G.M.T.

We would interpret these results as indicating that night-time conditions set in at least one hour earlier than usual on August 31. That echoes were not seen about 1830 G.M.T. is taken as evidence that there was no corpuscular eclipse, but it must be borne in mind that we are at all times arguing from incomplete data when discussing the Corner Brook results.

Discussion

It is not difficult to deduce the main conclusion from the results under consideration. For the Kennelly-Heaviside layer we can say with certainty

that the ionization in the path of the shadow decreased by at least 58% during optical totality. There was no indication of abnormal conditions about 1830 G.M.T., so that we conclude there was no particle eclipse in the shadow belt. The results from Corner Brook support these statements. In Newfoundland either there was no particle eclipse, or its effect was much smaller than that of the partial optical eclipse, the latter apparently being accompanied by a decrease in lower layer ionization.

The experiment is regarded as giving substantial proof of Appleton's theory that the predominant cause of lower layer ionization is the sun's ultra-violet light.

Ample corroboration of this statement is to be found in the work of other investigators during the recent eclipse, part of which has been summarized already from preliminary reports and appears in "Nature", September 10, p. 385 (1932). For example, trans-Atlantic signals from Rocky Point, N.Y., were received at Cupar, Baldock, and Dollis Hill in Great Britain. The frequency was 60 Kc., so that the lower layer is almost certainly the region involved. Although no important changes were noticed on the day of the eclipse at the two latter stations, Cupar receiving station gave a curve of signal strength variations which shows an abrupt decrease in strength between 2015 and 2018 G.M.T. followed by an increase which exceeds the normal value at 2030 G.M.T. The curve has added interest in that it shows no effect that could be ascribed to particles despite the fact that the transmission for the most part takes place inside the region of the predicted corpuscular eclipse. The exceptional increase in signal strength after the eclipse should be compared with the increase in ionization observed in Ontario shortly after totality.

This abnormal increase of ionization after the eclipse may be due, in part, to a local thunderstorm which occurred near Ottawa soon after totality. Professor Appleton finds that an increase of ionization actually does occur during thunderstorms as one would expect from C. T. R. Wilson's theory, and suggests it as a possible explanation here.

From the preliminary report it appears that the Bureau of Standards in Washington also measured ionic content of the lower layer and obtained results in close agreement with those described herewith, indicating "that the partial ultra-violet eclipse at Washington with a duration of about $1\frac{1}{2}$ hr. was accompanied by a slightly lagging reduction of something between 35 and 60% in the density of ionization in the Kennelly-Heaviside region".

The main results of other Canadian experiments described in the third paper of this series (9) are also in agreement with the conclusion just drawn, and we have no hesitation in ascribing the predominant cause of lower layer ionization to ultra-violet light from the sun.

For the upper layer our own results are not as decisive as those for the lower layer, which latter region indeed was our primary consideration. From the variation of equivalent height of the upper layer on two or three frequencies we would say that there was a reduction in the ionic content of the

Appleton layer about totality, with a subsequent increase to more normal values after the eclipse. Thus far the statements are definite and based on fairly reliable data.

More indefinite are some vague suggestions of an increase of effective height about 1830 G.M.T. Whether this is merely some chance variation, or whether it is a real "particle" eclipse, we are not prepared to say from these results only. The brief sketch of the results obtained at Cambridge, England, seems to bear out these remarks concerning a small particle eclipse, but the vagaries of the ionized layers are well known and more conclusive information is desirable. Furthermore, our own station in Ontario was situated beyond the western boundary of the predicted particle eclipse, and for the English station the particle eclipse ended about sunset so neither station was in a suitable location for observing the effect. Corner Brook, in a better situation than Vankleek Hill, reports that on 50 m. communication conditions underwent some modification, outside stations being heard more strongly than usual, or were heard when normally inaudible, but the evidence is not altogether conclusive. We must return the verdict of "not proven" on the point, when speaking of our own observations.

The equivalent height curve for 4.0 Mc. is a particularly bad one from which to draw inferences of ionic content, because the observed height varies most erratically on the other six days of observation; in fact the upper curve of Fig. 4 is no worse than for the other days. The curve for 3.16 Mc. is a little better and that for 2.73 Mc. is good, but on the debatable point of an effect at 1830 G.M.T. this last curve is useless since the signals are then reflected from the Kennelly-Heaviside layer.

Definitely it can be said that night-time conditions were much more closely approached during optical totality than at any other time of the day, which would indicate that the chief cause of ionization in the Appleton layer, as in the Kennelly-Heaviside layer, is ultra-violet light from the sun.

In presenting the results it was mentioned that on each one of the days August 28, 29 and 30, a blank period occurred during which it was impossible to obtain echoes on any frequency between 6.0 Mc. and 1.77 Mc. It is known that these days were magnetically disturbed days. Appleton and Naismith (6) have shown that, under such conditions, the ionization in the E region is more intense than usual. It seems extremely unlikely that, during the blank periods we speak of, the ionization of both layers should temporarily decrease to such an extent that even 1.77 Mc. penetrated the upper layer. Such an hypothesis would necessitate an enormous decrease in upper layer ionization. It is possible, however, that the "D" layer, noticed by Appleton (3), which is an ionized region below the E, normally capable of absorption but not of reflection of radio waves, was unusually intensely ionized, and the sky wave was so heavily absorbed on all our available frequencies that no reflections were obtained. The suggestion is put forward very tentatively as there is no decisive evidence to support it.

We conclude that the importance of ultra-violet light as the primary cause of ionization in the lower layer is established. Ultra-violet light is also

an important cause of upper layer ionization but its relative importance in this case cannot be deduced from the present experiments. There was no particle eclipse for the lower layer in Ontario.

Acknowledgments

The author is deeply indebted to many persons and organizations for assistance throughout the course of these experiments. The actual experimental work was done in co-operation with Messrs. W. B. Ross, J. C. Stadler, H. R. Smyth, and R. H. Prissick. After the preliminary tests in Montreal, Ross and Stadler went to Corner Brook, Newfoundland, while Smyth and Prissick assisted at Vankleek Hill. The author is very grateful to his coworkers as pressure of time necessitated particularly strenuous efforts.

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RADIO OBSERVATIONS ON THE UPPER IONIZED LAYER OF THE ATMOSPHERE AT THE TIME OF THE TOTAL SOLAR ECLIPSE OF AUGUST 31, 1932¹

By D. C. ROSE²

Abstract

This report contains the results from one of the three stations set up by the National Research Council of Canada to take observations on radio reflections from the ionized layers in the upper atmosphere during the total solar eclipse of August 31, 1932. The station concerned was located at Kingston, Ontario, and was approximately under the centre of totality for the upper or Appleton layer. Hence observations at this station were limited to the upper layer. The results from the other two stations at Corner Brook, Newfoundland, and at Vankleek Hill, Ontario, are reported in the paper immediately preceding (8).

The method was that developed by Breit and Tuve, in which a short pulse is transmitted, the reflection being received and its time delay recorded by means of a cathode ray oscillograph.

The results indicate that the ionization of the upper layer is caused by radiation (presumably ultra-violet light) from the sun. Whether or not this is the sole cause is uncertain because of the time lag in recombination of ions in the layer. A reduction in ionization of over 30% was noted.

A magnetic storm which occurred a few days before the eclipse made the results more difficult to interpret but gave some information of the effect of such a storm on the upper layer. It appeared to cause considerable instability in the layer and a somewhat lower ion content.

Introduction

In this paper the results of observations taken at Kingston, Ontario, on reflections of radio waves from the upper ionized layer of the atmosphere are given and discussed. The paper by Henderson (8) describes the results of observations taken at the time of the eclipse at Corner Brook, Newfoundland, and at Vankleek Hill, Ontario. The object of these experiments was to decide definitely whether or not the ionization in the reflecting layers, particularly the lower layer, was caused by radiation from the sun, such as ultra-violet light, or by corpuscles shot out from the sun. The two theories were introduced by Appleton and Chapman, respectively. The nature of the problem is well described in the early paragraphs of Henderson's paper and by Chapman (6) and by Miller (10), so little need be said about it here, except that a total eclipse of the sun offers an excellent opportunity to distinguish between the two possible phenomena. Although Chapman's theory suggests that only the lower, or Kennelly-Heaviside, layer is likely to be caused by particles, a knowledge of the behavior of the upper or Appleton layer under the conditions of a total solar eclipse, where solar sources of ionization are cut off relatively rapidly for only a short period, is also of great interest. Also the behavior of the reflecting layers in general had not hitherto been investigated in Canada. Hence an investigation of the upper layer in co-operation with the two stations mentioned above was necessary

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in order to obtain as complete a knowledge as possible of reflections of radio waves over the period during which observations are being reported.

Kingston was chosen as a site for observations on the upper layer because it was just under the central band of totality for a height of about 220 km. The results indicate that a location 50 to 70 miles farther west would have been nearer the desired location (under the central line of totality for the upper layer), as the layer at the time of the eclipse was found to be between 300 and 400 km. in height. However, such a location farther west should have a negligible effect on the results, because the shadow band was of the order of 100 miles wide and the effects of the eclipse on radio reception should not be very different for totality and for conditions a few per cent under totality.

Apparatus and Procedure

Transmitter

The method of measurement was that developed by Breit and Tuve (5). In this method the time lag is measured between the reception of a short signal coming directly along the ground, and the reception of the same signal reflected from the ionized layer in the upper atmosphere. The transmitting apparatus consisted of a short-wave transmitter developed by the Royal Canadian Signals and designated as their *M6* transmitter. It consists of a Hartley circuit using two *UX852* oscillator valves in parallel. It had an output of about 200 watts and was arranged to work at wave-lengths between 15 and 90 m. It was conveniently mounted for transportation and was self-contained in that it included its own rectifier power unit fed from 110-volt lighting lines. The keying operation was performed by interrupting the negative power lead.

For the present experiment a short signal of the order of one four-thousandth of a second was sent out 30 times per sec. The received signals appear to be of greater duration than one four-thousandth of a second probably because of the time required for oscillations to die down in the transmitter, or because the action of the receiver was too sluggish. The transmitter was controlled by a commutator which closed the key circuit for the required interval of time. The commutator was driven by a synchronous motor fed from the local 60-cycle power line. The wave-lengths were measured to an accuracy of better than 1% with a portable wavemeter loaned to us and calibrated by the Royal Canadian Signal Corps in Ottawa.

The transmitting aerial was that belonging to the Queen's University broadcasting station, the transmitter being set up in the radio laboratory of the Department of Electrical Engineering. The aerial appeared to work in a satisfactory manner at all wave-lengths used, though its natural period was in the broadcast band.

Receiving Equipment

The receiving equipment consisted of, first, a National *NC5* five-tube short-wave converter which was designed to convert wave-lengths from 15 to

200 m. into the broadcast band at 575 Kc.* The converter was a super-heterodyne type operated by alternating current. It was connected directly to a Stewart Warner T.R.F. eight-tube broadcast receiver, type R100. The audio-amplification in the broadcast receiver had to be altered to resistance-capacity coupling, as iron transformers would not respond properly to the short signal. However, after making the necessary alterations it was found that sufficient voltage to actuate the cathode ray oscillograph could be obtained directly from the detector. The output from the detector was connected through a potentiometer gain control directly to the vertical component plates of a von Ardenne cathode ray oscillograph.

The time axis or horizontal sweep of the cathode ray oscillograph was arranged by the usual method of charging a condenser through a high resistance until sufficient voltage is attained to break down a grid-controlled gas-filled valve (Western Electric Type 269A). The results would have been better if a linear time axis had been used by the introduction of a diode in the circuit, but owing to circumstances which made the time for preparation and testing of the equipment much too short it was impossible to arrange a linear sweep circuit in this case.

The receiving equipment was set up in the Physical Laboratory of the Royal Military College in Kingston, which is about a mile and a half from the transmitting station at Queen's University. The receiving aerial was hung between the roofs of the Physical and Engineering Laboratory buildings of the Royal Military College. It was from 20 to 30 ft. high and about 100 ft. long. The lead-in wire was connected from one end of the approximately horizontal section through a window to the receiving set.

Procedure

The apparatus was taken to Kingston on August 22 and set up as quickly as possible. This should not have taken long but owing to the fact that time had not been available to test all the equipment in Ottawa before moving it to Kingston some alterations had to be made, particularly in the time axis circuit on the cathode ray oscillograph. The circuit taken to Kingston and tried first was that recommended by Appleton and Builder (2), in which a condenser is discharged through a neon tube. In the present case a neon tube could not be found which always broke down at the same potential, so the arrangement had to be abandoned. The 269A valve gave much better results. The first oscillograms showing reflections were taken on August 23 using a 60-cycle wave as a time axis. The first run which gave satisfactory measurements of the frequency which penetrates the upper layer was obtained on August 26.

In the final arrangement of the time axis circuit, the circuit was coupled with the Ontario Hydro-electric 60-cycle power supply which was also feeding the synchronous motor, described above, used for keying the transmitter. This kept the time axis in synchronism with the incoming signal. This time

* Kilocycles per sec.

axis or horizontal sweep was calibrated periodically by changing the vertical sweep over from the receiver to the output of a General Radio beat frequency oscillator. This oscillator contains a vibrating reed by means of which one point on its scale can be calibrated. The rest of the scale was calibrated in Ottawa and found to be much more accurate than was needed. The frequency used for calibrating the time sweep was 1500 cycles per sec., so that one cycle corresponded to an equivalent layer height of 100 km.

The use of the time sweep circuit coupled with the a-c. power supply, as described above, made the a-c. power supply really the basis for time measurements, as the beat frequency oscillator had to be adjusted to make the approximately 1500-cycle wave (25 times the power frequency) stand still on the oscillograph screen. The inaccuracy in this process is the same as the inaccuracy in the frequency of the Hydro-electric power supply. However, on each occasion that the time axis was calibrated the frequency at which the beat frequency oscillator had to be set was noted, and it was found that for any observation the frequency of the power supply did not vary from 60 cycles per sec. by more than 1 or 2% and usually considerably less than 1%. The height measurements at their best involve possible errors greater than this amount.

It was intended that, after taking two or three days observations on the general behavior of the upper layer in the afternoon, two or three frequencies would be selected for height measurements. Observations would be taken on these frequencies, say, every 20 min. on the day of the eclipse and on the two days before and after the day of the eclipse. Between these 20-min. periods the frequency would be increased to find the minimum penetration frequency or the lowest frequency at which no reflection could be found. A private telephone line was established between the transmitting and receiving stations to facilitate frequency changes. However, due to a magnetic storm which started on August 27, and was particularly active on August 29 and 30, the plans were completely upset. The storm* had such an effect on the upper reflecting layer that it was impossible to select suitable frequencies for height measurements, or to find the penetrating frequencies representing an average undisturbed day, until after the eclipse.

Observations were taken on August 26, 27, 29, 30, 31 and on September 1 and 2. The runs were started about 1 p.m. E.D.S.T. (1700 G.M.T.**) and were continued until about 7 p.m. with slight variations in these times. On August 27 the results were rendered useless by a thunderstorm which was active for most of the afternoon. On Sunday, August 28, no records were taken, most of the day being spent in preparation for the rather strenuous continuous observations on the next five days. The activity of the magnetic storm mentioned above made it difficult to decide which of the seven days' observations should be taken as normal undisturbed days. The results of

* This storm also had a definite effect on the signal-strength measurements taken by the Canadian Marconi Company. (See the succeeding paper by Henderson and Rose (9).)

** 1700 G.M.T. means 17 hr. 0 min., Greenwich Mean Time.

runs taken on August 26, September 1 and 2 show similar characteristics, hence these are taken to represent normal undisturbed conditions. In spite of some remaining activity of the magnetic storm on August 31 there is but little doubt of the effect of the eclipse on the ionization of the upper layer.

Considerable trouble was experienced due to interference from various sources. A thunderstorm in the neighborhood of course makes observations useless as mentioned above. However, fortunately only one day's results were spoiled in this way. Local power distribution lines in the neighborhood of the Royal Military College gave some trouble, but this was eliminated promptly by the radio inspector of the Department of Marine for Kingston. Another type of interference which gave a pattern of the type shown in example No. 10 of Plate I gave trouble intermittently on all days on which observations were taken. A test arranged with a Kingston citizen showed that the trouble was probably due to a high frequency equipment used in medical treatment. It was, of course, impossible to locate all such machines in the city in the time available, but those in charge of the few that were well known were consulted and promised to use their machines only when absolutely necessary during the week of the eclipse. The result was that the trouble was not serious though sometimes for short periods it was annoying. By careful manipulation of the relative phases of the time sweep and the commutator controlling the transmitter, the peak representing the reflected signal on the cathode ray oscillograph screen could be made to come between two of the peaks caused by the interference. Hence the reflection could usually be detected with fair certainty in spite of the interference, but height measurements were sometimes impossible.

Results

Nature of the Upper Layer Apart from Eclipse Observations

Owing to the effect of the magnetic storm on the few days preceding the eclipse, one cannot draw curves representing either layer heights or penetration frequencies displaying conditions on an average undisturbed day and compare them with similar curves on the day of the eclipse. Of all observations taken, those on September 1 and 2, and a few obtained on August 26, are the only ones that show any similarity. These have been taken to represent average undisturbed conditions though they show a phenomenon which, as far as the author is aware, has not been noted before, *i.e.*, the appearance of a critical frequency at which no echo appeared, though echoes appeared from the same layer at both lower and higher frequencies. The existence of this phenomenon was not noticed until the day after the eclipse. The usual procedure in taking observations was to start at some frequency at which an echo was fairly certain, say 4.61 Mc.* (65 m.) and proceed to reduce the wave-length by steps of $2\frac{1}{2}$ m. until the echo disappeared. Then the first point at which, after careful visual observation of the oscillograph, no echo was found was taken as the penetration frequency and the results plotted

* Mc. means megacycles per second.

as in Figs. 1 and 2. When the critical point was found, the wave-length was not usually lowered further. Hence in early observations this reflectionless frequency band may have been overlooked, though had it not been for the

disturbed conditions, assumedly due to the magnetic storm on the few days before the day of the eclipse, this reflectionless band would, no doubt, have been discovered earlier. The best example is shown in the results for September 2 (Fig. 2).

Examining the results for September 2, it is seen that, from the time observations started until about 2000 G.M.T. the penetration frequency was about 5.7 Mc. (52½ m.) but at the same time there was a band about 5.00 Mc. (60 m.) where no echo could be found. This condition altered at about 2000 G.M.T. and from then until the observations ceased echoes occurred at all frequencies below the penetration frequency, but

there was a distinct difference in the appearance of the patterns at a frequency of 5.00 Mc. At this frequency the echo was split into several components, while echoes at frequencies below and above 5.00 Mc. were single sharp peaks. The points where these split echoes were found are marked with an X in Fig. 2, and the periods when the reflectionless band appeared are indicated with a broken line. Examples of the oscillograms showing the split and sharp echoes are shown in Plate I, examples Nos. 11 to 14. No. 11 shows a clear single echo. The frequency at which this oscillogram was taken was 5.46 Mc. (wave-length, 55 m.). Nos. 12 and 13

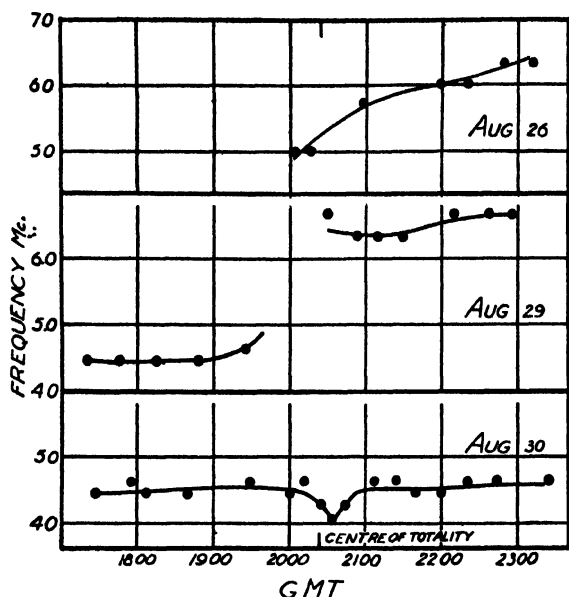


FIG. 1. Penetration-frequency curves for August 26, 29 and 30.

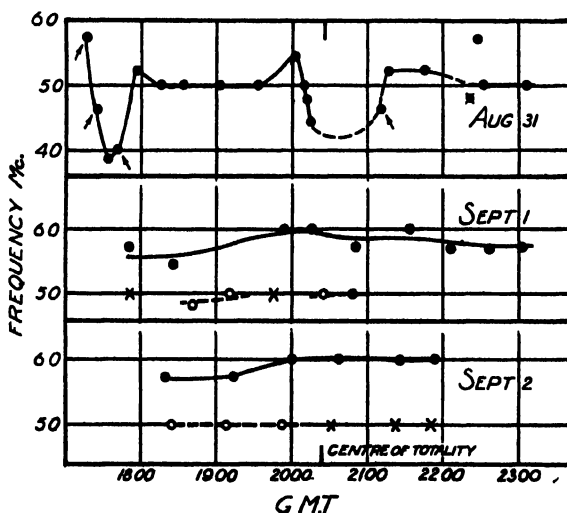
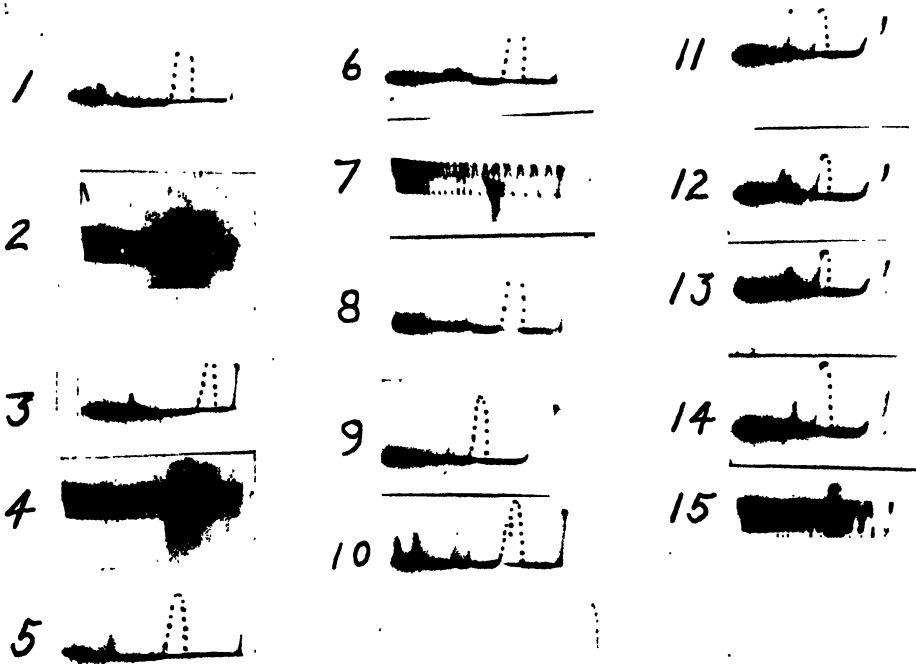


FIG. 2. Penetration-frequency curves for August 31, September 1 and 2.



Examples Nos. 1, 3 and 5 show echoes taken on August 29 at a frequency of 4.29 Mc. at 1839, 1852 and 1905 G.M.T., respectively. They are typical examples of unusually high equivalent heights found on that day. No. 2 is the time axis calibration for No. 3, and No. 4 the calibration for No. 5.

Examples Nos. 6 to 10 were taken on the day of the eclipse. No. 6 was taken at 2016 G.M.T. at a frequency of 4.14 Mc. Note the multiple splitting and haziness of the echo due to the constantly changing intensity of the components. No. 8 was taken a few minutes later and shows the effect of a thunderstorm. No. 9 was taken at 2140 G.M.T. at a frequency of 5.00 Mc. It shows a typical echo under steady conditions. No. 10 was taken one minute after No. 9 and indicates the effect of interference probably due to a high frequency machine for medical treatment (see page 19). No. 7 represents a time axis calibration for Nos. 6 and 8.

Examples Nos. 11 to 15 were taken on September 2. They are arranged to show the different type of echo at the frequency which, earlier in the afternoon was found to be reflectionless. No. 11 shows a sharp single echo at a frequency of 5.46 Mc.; Nos. 12 and 13 are examples of the multiple split echo at a frequency of 5.00 Mc. at which frequency no echoes were found before about 2000 G.M.T. (see page 20). Example No. 14 is a typical echo at a frequency of 4.61 Mc. No. 15 is a time axis calibration suitable to Nos. 11 to 14. Nos. 11 to 15 were taken between 2100 and 2200 G.M.T. on September 2nd.

The reproduction of the ground signal is not very good as the oscillograms showed it very faintly. To make it show up better the ground signal has been emphasized by a row of dots where necessary.

are examples of echoes at a frequency of 5.00 Mc. (60 m.). These two examples are the clearest obtained during all observations and are better than the average oscillogram. However, they show no great exaggeration of the effect noted, No. 14 represents a reflection at a frequency of 4.61 Mc. (65 m.). Again a single sharp echo is seen with a very weak echo due to double reflection.

On September 1 the results were not as definite as on September 2, but the same peculiarity was noted at about the same frequencies. Throughout the early part of the afternoon the reflectionless band appeared intermittently as indicated by the broken line and the split character of the echoes at intermediate points marked X was noted. Later in the afternoon the oscillograms showed the split echoes occurred at a frequency of 4.61 Mc. (65 m.), while at higher frequencies the echo was sharp. Frequencies lower than 4.61 Mc. were not tried, hence no points indicating split echoes are included in the curve after 2100 G.M.T. On August 26 this same phenomenon may have existed, as only two good points were obtained on the penetration-frequency curve before the time when reflections appeared consistently at 5.00 Mc., namely, 2000 to 2100 G.M.T. Higher frequencies were not examined at the times represented by these two points but if they had been the phenomenon indicated by the curves for September 2 might have been observed. On August 29, Fig. 1, the same may have been the case but if so the reflectionless frequency was lower. In the early part of the afternoon frequencies higher than 4.61 Mc. were not examined until shortly after 2000 G.M.T., as it was assumed that if no reflection occurred at 4.44 Mc. and at 4.61 Mc. the penetration frequency had been passed and it was pointless to proceed to higher frequencies. On August 30 the penetration frequency remained abnormally low all afternoon and it is unlikely that the reflectionless band discussed above occurred at all, because several times during the afternoon frequencies up to 5.00 Mc. were observed, the penetration frequency remaining at about 4.44 to 4.61 Mc. On August 31, the day of the eclipse, the reflectionless band was not observed though it may have existed at about 5.00 Mc., as observations were taken at higher frequencies only if a reflection occurred at this frequency.

The statement that this band exists, at which no reflection takes place, while echoes from the same layer are found at higher and at lower frequencies, is introduced with considerable reservation because the existence or lack of existence of an echo depends on the type of receiver and its surroundings. The critical penetration frequency of the upper layer has an arbitrary factor depending on the sensitivity of the receiver. In the present case observation of the cathode ray oscillograph screen appeared to leave but little doubt as to whether or not there was an echo. Though in many cases it was very small it was only rarely that the author had any doubt whether or not the echo existed. This would indicate either that the upper layer reflects an easily measurable signal or none at all; or that the receiver would indicate either an easily observable signal or none at all. As the sensitivity of the receiver

and oscillograph for weak signals was not studied in detail the arbitrariness in the penetration frequencies remains.

Another possible explanation of the existence of a reflectionless band might be that the energy in the echoes was absorbed by some resonance effect in the steel frame buildings in which the receiver was located, or between which the aerial was hung, or in the receiver itself. Similarly selective absorption may have been present in the neighborhood of the transmitter. Such effects, if they existed, did not show up in the reception of the ground wave. This point however is not very significant as the ground wave was very strong.

Neither the effects of neighboring buildings nor the lack of sensitivity of the receiver could account for the splitting of the reflected signal at certain frequencies while a sharp single echo was found at both higher and lower frequencies. That reflections at frequencies both higher and lower than the so-called reflectionless band come from the same region or nearly the same region is clear from the equivalent height curves for September 1 and 2 (Fig. 3), where heights for reflections at frequencies of 5.46 Mc. (55 m.) and 4.61 Mc. (65 m.) are shown. The reflectionless band was usually at 5.00 Mc. (60 m.). The differences in height are usually less than 100 km. and could be accounted for easily from the shape of the height-frequency curve given by Appleton and Naismith (3). The equivalent heights of reflections at 5.00 Mc. where found are rather erratic, and owing to splitting and general instability of the echoes are not sufficiently reliable to be shown by a curve which would mean anything. The heights are always measured from the first point of deviation of the ground signal from the time axis, to the corresponding point on the reflected signal. When the reflected signal is considerably split, the intensity of the various components is constantly varying, giving an effect on the oscillograph best described as "boiling". Very often the component of shortest delay may disappear for a few seconds. If a photograph is taken at this time the measured height may be abnormally high.

This frequency at which either no reflection from the upper layer appears or the reflected signal is split into several components should be investigated further. If after local effects are eliminated it still exists it may represent selective absorption in a lower layer, either the E layer or an absorbing but non-reflecting D layer as suggested by Appleton (1). The pattern presented when the reflection is split is not usually the simple form discussed by Eckersley (7) and by Appleton and Ratcliffe (4) as being the result of double refraction due to the effect of the earth's magnetic field. While sometimes only two peaks appear, usually several are seen constantly changing in intensity with no apparent relation to each other. This made such echoes difficult to photograph as exposures of about one-half second had to be used.

Effect of the Eclipse

The curve for August 31 in Fig. 2 and the curve in Fig. 4 represent the penetration frequencies and the effective height for a frequency of 4.61 Mc. respectively. It has been assumed, for reasons given above, that the results

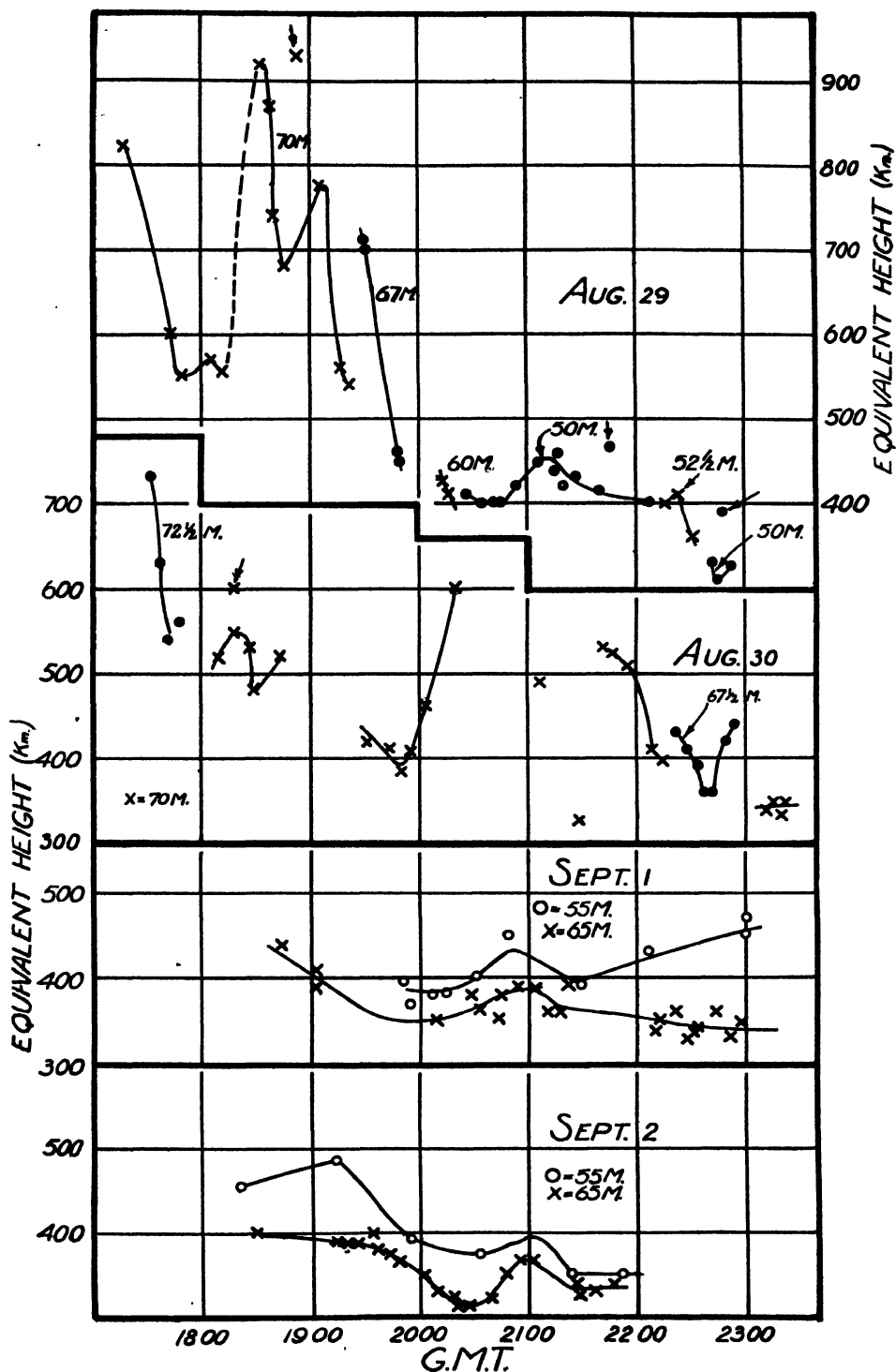


FIG. 3. Equivalent heights of the upper reflecting layer at various wave-lengths on August 29 and 30, and September 1 and 2.

of September 1 and 2 represent a normal undisturbed day, so the results of August 31 should be compared with these. First examining the relatively flat part of the curve representing penetration frequencies for August 31 between 1800 and 2000 G.M.T., it is seen that conditions do not appear to be quite like those on September 1 and 2. The penetration frequency during this period is somewhat lower on the day of the eclipse than on a normal undisturbed day, though as mentioned above the penetration frequency indicated may not have been the lower limit due to the possible existence of the reflectionless band. If this is the case the curve for the early part of

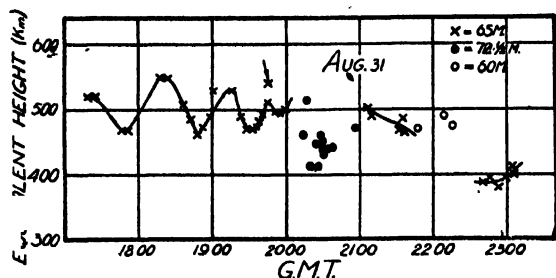


FIG. 4. Equivalent heights of the upper reflecting layer on August 31.

the afternoon, neglecting the sharp depression between 1700 and 1800 G.M.T., compares well with the broken line on the curve for September 2. The sharp depression between 1700 and 1800 G.M.T. will be discussed later. Until about 15 min. before optical totality (approximately 2024 G.M.T.) the conditions were normal when at 2010 G.M.T., while working at a frequency of 5.00 Mc. the reflection suddenly disappeared and the penetration frequency dropped rapidly, a steady echo being found at 4.14 Mc. ($72\frac{1}{2}$ m.). Here the echo was split into a large number of peaks which were changing in intensity too rapidly to obtain a good photograph. A reproduction of such a reflection is shown in example No. 6, Plate I. The time delays of the most distant peaks found represent equivalent heights up to 800 or 1000 km. The broken line in the penetration-frequency curve in Fig. 2, August 31, is an estimate from visual observations of what was happening. Changes came too suddenly to obtain good points on the curve until about 2120 G.M.T., though one intermediate point at 2110 G.M.T., marked with an arrow head, is given, as the echo at this point appeared as though the frequency was very near the penetration point. At about 2020 G.M.T. a thunderstorm which could be seen some miles down the St. Lawrence river became active, making it additionally difficult to obtain oscillograms during this critical period.

Conditions from 2120 G.M.T. until observations ceased, appeared to be quiet, except for a short period, indicating a penetration frequency of about 5.00 Mc. rather than about 6.00 Mc. as on September 1 and 2. The exception occurred a little before 2230 G.M.T. when echoes at 5.00 Mc., which had been coming steadily for some time, seemed to disappear and a small echo appeared at 5.46 Mc. (55 m.) disappearing again at 5.72 Mc. ($52\frac{1}{2}$ m.). This was the first discovery of the reflectionless band discussed in the previous section and even then its existence was not apparent until notes were carefully examined the following morning. The points are marked on the curve.

The nature of the transient penetrating frequencies at the time of optical totality is evidence that radiation from the sun, presumably ultra-violet light, has a decided effect on the ionization of the upper reflecting layer. The curves representing the variation in height are not so conclusive, largely because the unstable state on the few days before the eclipse, lasting until the day of the eclipse, has left an insufficient knowledge of normal conditions for comparison. The equivalent height at a frequency of 4.61 Mc. (65 m.) is somewhat higher on the day of the eclipse than on the two succeeding days, which have been taken as normal. During the eclipse period, echoes at this frequency disappeared, though the equivalent height of the layer for a frequency of 4.14 Mc. ($72\frac{1}{2}$ m.) does not appear to be abnormal.

The relation between ionization and penetration frequency used by Henderson (8), indicates that the density of ionization is proportional to the square of the penetration frequency. In the present case the stoppage of radiation from the sun by the moon reduced the density of ionization of the upper layer by 30% or more.

An effect due to the corpuscular eclipse predicted by Chapman (6) should be at its maximum at about two hours before optical totality. That would be between 1800 and 1900 G.M.T. The results on the day of the eclipse indicate no abnormal effects at this time, though there was an abnormality between 1700 and 1800 G.M.T. This peak shown on the penetration-frequency curves for August 31 is taken to be due to the continued activity of the magnetic storm, rather than an indication of an effect due to a corpuscular eclipse, for two reasons. First, it is not unlike a similar abnormality on the preceding day; second, not only does it occur an hour too soon, but Kingston is well outside the region where any corpuscular eclipse was predicted. (See the map on page 35.)

If the above interpretation of this abnormality between 1700 and 1800 G.M.T. is accepted, the results definitely indicate that the ionization of the upper reflecting layer is caused to a considerable extent by radiation from the sun, though it will be seen in the next paragraphs that there are other phenomena, presumably magnetic storms, which have a considerable effect on the nature of this layer.

Effect of Magnetic Storms

The unstable effects noted on the few days before the eclipse are attributed to the magnetic storm, because it is known that magnetic storms have an effect on radio reception and a magnetic storm was reported as starting on Saturday, August 27, and being at its height on August 29 and 30. (See report of the Canadian Marconi Company on trans-Atlantic reception in the succeeding paper (9)). The effect of this magnetic storm on the upper reflecting layer of the earth's atmosphere is indicated by abnormally low penetration frequencies and unstable effective heights. An examination of the penetration frequency curve for August 29, Fig. 2, shows that early in the afternoon the penetration frequency was abnormally low. At about 1930 G.M.T. observations were stopped for about 25 min. and when started

again it was found that a transition had taken place, the penetration frequency having risen to such an extent that a point was not obtained on the curve until about 2030 G.M.T. From this time until observations ceased the penetration frequency was found to be about 6.5 Mc. This value is slightly higher than that for September 1 and 2, the days which have been assumed to be normal.

It may be that the first part of the curve for August 29 is not the true penetration frequency but represents a reflectionless band of the type found on September 1 and 2, occurring at about 4.5 Mc. instead of 5.00 Mc. as on the latter days. The true limit may have been missed because, once it was noted with certainty that the reflection had disappeared, observations were not made at higher frequencies. On August 30 the penetration frequency was abnormally low all afternoon with an exceptionally low point due to some disturbance at about 2030 G.M.T. On this day there was no evidence of a reflectionless band as frequencies from 5.00 Mc. to 4.00 Mc. were observed periodically throughout the run.

The existence of the discontinuity in the penetration-frequency curve for August 29 and the depression in the curve for August 30 were rather disconcerting in view of the fact that they came very near the time of totality. Indeed there is nothing to indicate that the depression in the penetration-frequency curve for August 31 at the time of the eclipse was due to the eclipse rather than the magnetic storm, except that conditions on the eclipse day were more nearly normal and the time and length of period of the disturbance coincided with the expected effect, when one considers that there should be a lag of several minutes due to the slow rate of recombination of ions in the reflecting layer. The depression in the penetration-frequency curve for August 31 between 1700 and 1800 G.M.T. is considered to be due to a further vagary of the magnetic storm. The points marked with an arrow on this curve are somewhat uncertain because a rapidly changing penetration frequency made it impossible to obtain points quickly enough.

The height measurements on August 29 and 30 are very erratic. Owing to the abnormal penetration frequencies, frequencies which could be compared with those for the normal days September 1 and 2 were not used. Hence in the equivalent height curves in Fig. 3 for August 29 and 30 an attempt was made to plot equivalent heights at frequencies slightly lower than the penetration frequency (usually $2\frac{1}{2}$ or 5 m. longer wave-length). The wave-lengths at which observations were taken are given on the curves in each case. Blank periods occur where information was lacking or where the penetration frequency was changing too rapidly for a series of observations at any one wave-length. Sometimes, owing to a lacking first component of a split reflection, exceptionally high equivalent heights are recorded. Such points are marked with an arrowhead on Fig. 3.

Summarizing the results of August 29 and 30 it can be said that the magnetic storm appeared to produce periods during which the ionization in the upper layer was abnormally low and the equivalent heights exceptionally high,

heights around 900 km. often being recorded. A sample of some of the records is shown in Plate I, examples Nos. 1 to 5.

Summary of Conclusions

The conclusions drawn from observations on reflections of radio waves from the upper ionized layer of the earth's atmosphere are that this ionization is caused at least in part by radiation from the sun, presumably ultra-violet light. This was demonstrated by the fact that about 15 min. before totality the penetration frequency (the lowest frequency which goes through the layer without reflection) dropped considerably, coming up again to its normal value at about three-quarters of an hour after totality. The lag in the effect or the lack of symmetry with respect to time, about the centre of totality, was expected, due to the time required for recombination of ions as the sun's rays were cut off and the forming of the layer as the sun's rays reach it again. This lag also tends to make it uncertain whether or not the ionization of the upper layer is entirely due to radiation from the sun. A decrease in ionization of over 30% was observed.

During the three days before the eclipse a magnetic storm which was known to be in progress had very noticeable effects on the upper ionized layer. The effect was a reduction in the density of ionization and unusually high but very unstable equivalent heights.

On the two days after the eclipse, which, owing to the effect of the magnetic storm had to be considered as normal undisturbed days, a new phenomenon was noted. At certain times of the day there appeared to be a frequency at which no echo was found, while echoes from the same region appeared at both lower and higher frequencies. When an echo did appear at this so-called reflectionless frequency it was split usually into several components, while echoes at lower and higher frequencies usually consisted of a single clear component. Lack of measurable echoes at a certain frequency might be due to local causes but no explanation is given for the selective splitting.

Acknowledgment

It was originally intended that the station in Kingston should have been in charge of Lieutenant-Colonel W. A. Steel, assisted by the author. About two weeks before the eclipse Colonel Steel was called to the International Radio Conference at Madrid but most of the preliminary planning, collecting and testing of equipment, was carried out by him. The author wishes to express his thanks to Colonel Steel for the way in which the preliminary work was completed, leaving little to be done but the setting up of the equipment in Kingston and the taking of the observations. Thanks are also due to Queen's University, particularly the Electrical Engineering staff; and to the Royal Military College, particularly the staff of the Physics Department, for the use of their laboratories, and their assistance. The observations were taken with the assistance of Staff-Sergeant C. M. Wright of the Royal Canadian Signals and Mr. Roy Burstow of the National Research Labor-

atories and thanks are due to them for the reliable way in which they carried out the work. Thanks are also due to the Associate Committee on Radio Research of the National Research Council of Canada under whose jurisdiction all radio observations were carried out.

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FADING AND SIGNAL-STRENGTH MEASUREMENTS TAKEN DURING THE SOLAR ECLIPSE OF AUGUST 31, 1932¹

BY J. T. HENDERSON² AND D. C. ROSE³

Abstract

This paper contains the results of observations on signal strength and fading, taken during the total solar eclipse of August 31, 1932, by the Canadian Marconi Company, the Northern Electric Company, the Marine Department of the Canadian Government and l'Ecole Polytechnique, Montreal.

The Canadian Marconi Company at Yamachiche took signal-strength observations on trans-Atlantic and American short-wave stations. The Northern Electric Company measured field strengths in Montreal from the Ottawa station in the broadcast band (600 Kc.). The stations of the Marine Department in the Hudson Strait and Hudson Bay regions and also in the Newfoundland and Nova Scotia region took notes on short-wave reception from Ottawa and direction-finding bearings on specified nearby stations. L'Ecole Polytechnique had a receiving station at Rigaud, Quebec.

The results of observations on short waves indicate no effect at the time of the predicted corpuscular eclipse, but a definite night effect at the time of optical totality. Direction-finding stations and observations on the broadcast band report no effect.

This report embodies the results of the radio observations made during the eclipse by the Canadian Marconi Company, the Northern Electric Company, l'Ecole Polytechnique, Montreal, and the radio stations of the Marine Department of the Canadian Government. The writers' present herewith the individual reports sent to Dr. A. S. Eve, the chairman of the Radio Eclipse Subcommittee of the National Research Council. The reports received are reproduced wholly or in part and such conclusions as appear to be justified are drawn.

Short-wave Reception across the Atlantic

The report received from the Canadian Marconi Company on reception across the Atlantic and from some stations in Canada and in the United States is best explained by publishing it as it was received:—

CANADIAN MARCONI COMPANY

REPORT ON SIGNAL-STRENGTH OBSERVATIONS DURING SOLAR ECLIPSE AUGUST 31, 1932

Program

It was arranged to take signal-strength observations during a period of four hours on the day of the eclipse, and during the same period for the preceding two days as well as the day after the eclipse, the observations to commence daily at 1830 G.M.T. and end at 2230 G.M.T. The time of totality of the eclipse at Yamachiche, P.Q., was approximately 2024 G.M.T.

¹ *Manuscript received December 9, 1932.*

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Observations

The observations were carried out at the Marconi trans-Atlantic receiving station at Yamachiche, P.Q. This station is located well within the path of totality. The stations observed included European trans-Atlantic telegraph and telephone stations as well as a few North American stations operating in the short-wave band.

The measurements were made in relative values calibrated in decibel gain or loss.

Receiving Conditions During Tests

Unfortunately, receiving conditions were poor during the first two days of the tests and all trans-Atlantic circuits observed were affected by a magnetic storm which had been in process since Saturday, August 27.

During August 29 and 30 the effect of the magnetic storm was sufficiently serious to prevent observations of any value. Conditions during August 31, the day of the eclipse, were moderately good.

On September 1 a local electrical storm occurred during the four-hour period and observations were again impossible.

Results of Observations

Table I gives the relative values of the observations taken on various short-wave stations during the four-hour period on the day of the eclipse.

The field intensity of most of the short-wave European transmissions showed no wide variation during the four-hour period. Comparisons made between the results of these observations and the observation records kept daily at the receiving station on the majority of these stations show that the relative strengths as given in Table 1 are, in general, normal for the particular time of the day and for the particular receiving conditions at the period.

In the case of Station WDD, located at Long Island, New York, U.S.A., our records show that signals from this station usually fall in strength at approximately the same time as the decrease in intensity occurred on the day of the eclipse.

The reception from Station CGA at Drummondville on 62.71 m. showed no change in signal strength during the four-hour period on August 31. The distance between this station and the receiving station is only of the order of 30 miles. The observations were taken on the ground-ray signal.

In the case of Station VE9GW located at Bowmanville, Ontario, the observations show a large fall in signal strength on the day of the eclipse, commencing at 2005 G.M.T. and continuing until 2030 G.M.T. A slow increase in intensity then occurred. Observations made on this station from 1700 to 0200 G.M.T. on several days during September when receiving conditions were normal showed no such decrease in intensity occurring at that particular period. An average of the measurements taken on these days gives the following results:—

1700 G.M.T.	+	7 D.B.	2200 G.M.T.	+	18 D.B.
1800	+	11	2300	+	18
1900	+	13	2400		Nil
2000	+	15	0100		Nil
2100	+	17	0200		Nil

TABLE I

SIGNAL-STRENGTH OBSERVATIONS, SOLAR ECLIPSE, AUGUST 31, 1932, TAKEN AT
YAMACHICHE SHORT-WAVE RECEIVING STATION

DB VALUES ARE ABOVE OR BELOW A REFERENCE LEVEL OF SIX MILLIWATTS (ZERO LEVEL)

G.M.T.	GBB 13585 Kc.	GLH 13535 Kc.	GNH 11580 Kc.	FTL 9970 Kc.	GOK 9260 Kc.	GLK 8005 Kc.	WDD 7510 Kc.	VE9GW 6095 Kc.	CGA 4765 Kc.
1830	—	-10	-10x	24	—	-10	18	8	8
1835	—	-7	-10x	14	—	-6	18	8	8
1840	—	-8	-10x	24	4	-6	18	8	18
1945	2	-9	-10x	22	—	-10	18	8	18
1950	-19	-9	—	22	-2	-6	18	8	14
1855	-20	-9	-10x	14	-6	-10	18	6	16
1900	—	-6	-10x	14	-6	-10	18	6	16
1905	—	-6	—	14	-4	Stops trans- mit- ting	18	8	16
1910	-10	-8	—	14	-10		18	6	16
1915	-18	-10	—	14	-8		18	6	18
1920	-18	-10	-10x	16	-4		18	6	16
1925	-8	-9	-10x	16	-2		18	6	18
1930	6	-9	-10x	16	0	-8	14	6	16
1935	2	-6	-8	10	0	-8	14	8	14
1940	0	-6	-10x	16	2	-10	14	6	16
1945	4	-7	-10x	20	2	-10	0	8	16
1950	4	-5	-10x	16	0	-10	0	8	18
1955	6	-6	-10x	16	2	-10	0	8	16
2000	4	-4	-10x	16	0	—	-7	10	18
2005	6	-2	-10x	16	4	4	-18	-2	18
2010	6	-1	-10x	20	8	4	-7	-2	20
2015	6	-1	-10x	20	14	12	-7	-6	16
2020	6	-2	-10x	20	14	18	-7	-8	22
2024	—	-2	-10x	—	—	—	-7	—	—
2025	6	—	—	22	14	18	-7	-16	22
2028	—	—	—	—	—	—	0	—	—
2030	6	-2	-10x	22	14	18	6	-20	20
2035	8	-2	-10x	22	14	18	6	-16	18
2040	8	-2	-10	16	Stops trans- mit- ting	14	10	-10	16
2045	—	0	-10	16		10	10	-2	16
2050	4	0	-10	16		12	10	-2	16
2055	2	2	-9	16		6	10	-4	20
2100	0	2	-9	22		12	10	-2	20
2105	-4	2	-9	22	8	12	10	2	18
2115	-2	2	-10x	22		18	10	4	22
2125	—	1	-10	22	12	18	18	4	22
2135	—	1	-10	22	—	18	Change wave- length	4	22
2145	—	—	-9	20	—	12		4	20
2155	—	—	-10x	16	—	12		4	18
2205	—	—	-10x	16	—	12		4	18
2215	—	—	—	16	—	12		8	12
2225	—	—	—	20	—	12		4	12

NOTE: -10x indicates less than -10 DB. Plain figures are plus.

GBB Rugby, England. 13585 Kc. 1500 Cycle tone.
GLH Dorchester, England. 13535 Kc. High speed telegraphic traffic to N.Y.
GNH Grimsby, England. 11580 Kc. High speed telegraph to Australia.
FTL St. Assise, France. 9970 Kc. Telegraph.
GOK Bodmin, England. 9260 Kc. 1000 Cycle tone.
GLK Dorchester, England. 8005 Kc. High speed telegraph to New York.
WDD New York. High speed telegraph to Yamachiche.
VE9GW Bowmanville, Ontario. Musical program.
CGA Drummondville, Quebec. 1000 Cycle tone.

This station continues operating up to 0300 G.M.T. but is not received at Yamachiche Station from 2400 G.M.T. onwards, possibly due to an increase in skip distance. Normally, therefore, the results produced by the so-called "night effect" on reception of Station VE9GW at Yamachiche receiving station is a complete fade-out of signals.

Summary

The occurrence of either the corpuscular or the optical eclipse gave little or no effect on reception, carried out at the Marconi station at Yamachiche, P.Q., of transmissions received from various European stations operating in the short-wave bands.

Reception from Station VE9GW located at Bowmanville, Ontario, approximately 336 miles from the receiving station, showed a decided drop in field intensity occurring 19 min. before the time of the optical solar eclipse and continuing until 6 min. after totality. A gradual increase in intensity to normal was then observed.

No such similar occurrence is experienced normally on transmissions from this station, except during the night period, when signals completely fade out.

Reception on the Broadcast Band between Ottawa and Montreal, carried out by the Northern Electric Company

The report of the Northern Electric Company is as follows:—

REPORT ON ECLIPSE MEASUREMENTS BY THE NORTHERN ELECTRIC COMPANY

At the request of Dr. Eve, Chairman, Associate Committee on Radio Research of the National Research Council of Canada, measurements were made at the Shearer Street plant of the Northern Electric Company in Montreal to study fading on the day of the total solar eclipse and for several days previous to, and on the day following, the eclipse.

Inasmuch as the centre of the shadow during the eclipse in the 100-km. Kennelly-Heaviside layer was located about midway between Montreal and Ottawa, it was thought that radio waves originating in Ottawa might be reflected by this layer so as to be received in Montreal and observed with regard to fading. Furthermore, the shadow in the 200-km. Kennelly-Heaviside layer being beyond Ottawa, reflection effects from this layer would not be involved.

Beginning on Saturday, August 27, and ending on Thursday, September 1, a daily record on a recording milliammeter was made at Montreal of the field strength of CNRO, the 500-watt broadcasting station, at Ottawa, of the Canadian National Railways. These measurements were made daily from 1 p.m. to 9 p.m. standard time. The results obtained on the days preceding the day of the solar eclipse failed to show any appreciable change in signal strength between day and night radio reception. It was considered that this was due to the predominance of the ground wave for the transmission distance

(about 170 km.) and transmission frequency (600 Kc.) involved, so that substantial variation, if present, in the downcoming waves did not appreciably affect the total intensity of received signal. This stability of received signal for day and night reception indicated the unlikelihood of any appreciable variation being observed in signal strength on the day of the eclipse. This surmise proved to be correct for, on the day of the eclipse, the record was substantially the same as for the days preceding and on the day following the eclipse.

Incidentally, an attempt was made to record the signal strength of CKCO, a 100-watt radio station at Ottawa transmitting on 890 Kc. It was thought that this station, being on a shorter wave-length, would have a relatively larger downcoming wave component, but unfortunately the power output of this station was not sufficient to permit of distinct reception above the prevailing noise level. Accordingly, no useful data could be obtained from signal-strength measurements on this station.

The apparatus set-up used to measure the field strength variations of CNRO is roughly outlined as follows: A loop antenna consisting of 15 turns was mounted on the roof of the Shearer Street plant, and was connected to measuring apparatus located in the shielded room of the Radio Laboratory by means of a shielded pair. The loop was tuned to resonance by means of a variable condenser and the shielded pair was connected to $\frac{1}{4}$ turn tapped off from the centre of the loop. An attenuator and a coupling circuit were connected in circuit between the incoming shielded pair and the grid of the first tube of the short-wave tuned radio-frequency radio receiver used in the set-up. A milliammeter inserted in the plate lead of the automatic gain control circuit of the radio receiver furnished an indication of the variation in received signal strength, while a recording milliammeter in series with this meter furnished a permanent record of the signal variations as a function of time. A signal generator was available for alternative connection to the receiver input in order to provide a signal of known intensity: this alternative connection was involved in a determination of the received signal strength in microvolts per metre corresponding to the readings of the recording milliammeter. The received signal strength from CNRO was found to be approximately 600 microvolts per metre.

Direction Finding and Short-wave Reception Observations taken at Various Stations in Northern and Eastern Canada

The program of observation undertaken by the Marine Department stations was as follows: from August 29 to September 2 inclusive, direction-finding signals were to be sent out from Nottingham Island and Canso daily between 1500 G.M.T. and 2300 G.M.T. Two-minute signals were sent out on the hour and every 15 min. throughout the period with the following variations: on August 31 a five-minute test was to be sent out at 2000 and 2040 G.M.T. and continuous signals were to be sent between 2010 and 2035 G.M.T. The wave-length of signals used was 800 metres.

Stations in the Hudson Bay and Northern regions were to take DF bearings on Nottingham Island and stations on the east coast on Canso. Besides this a short-wave and long-wave watch was to be kept on signals from Ottawa (22-metre band). Stations were particularly asked to watch for night variations.

No pronounced night effect on direction-finding observations is reported from any of the stations, either in the northern (Hudson Bay and Hudson Strait) or the eastern (Nova Scotia and Newfoundland) regions at the time of the optical eclipse or the predicted corpuscular eclipse. It should be noted that atmospheric conditions were very heavy on the day of the eclipse due to local thunderstorms around the gulf of St. Lawrence.

Stations reporting DF observations are:—

Cape Hopes Advance	}	From Nottingham Island.
Churchill		
Resolution		
St. Paul Island	}	From Canso.
Cape Race		
Yarmouth		
Chebucto Head		
St. John		

These (except Churchill, which is on the southwest shore of Hudson Bay) are marked on the map (p. 35).

On the other hand, reports from Marine Department stations on short-wave receptions show more definite results. Cape Hopes Advance reports as follows: "Hopes Advance nil heard till 2034 signal strength R 2 slight fading. McLean 2005, R 4, then gradually decreased until at 2014, R 1 to zero, then gradually increased till 2023 when R 7, again decreasing till 2030 when R 3 to R 2, then increasing rapidly to Rs at 2035. Considerable QRN noticeable during first part of test decreasing at 2020". (Time G.M.T.) Note the violent fading after the time of the optical eclipse. (Eclipse at this point was 90% total at 1955 G.M.T.)

The above report covers reception from Ottawa on 22-metre band by Cape Hopes Advance and SS. N. B. McLean in position indicated on map. The signal-strength levels are conventional levels from 1 to 10 and are estimates made by operator from the loudness of the signal in the phones.

The following is reported from the Experimental Farm in Ottawa:—

CNR Rabat Morocco, 23-Metre Band. Normally R 5 to R 3 during early afternoon, gradually increased in strength reaching R 9 plus for about 10 min. between 3.30 and 4.20 p.m. and gradually diminished until R 5 about 5 p.m.

IAC Coltano Italy, 23-Metre Band. Normally R 4 to R 3 showed a similar effect to Rabat also reaching R 9 maximum between 3.20 p.m. and 4.20 p.m.

KPH Bolinas Col., 23-Metre Band. Usually not heard until 4 p.m. when he begins coming in in R 2, was heard working R 5 to R 4 at 3.03 p.m. and continued throughout afternoon.

Times indicated are Eastern Daylight Saving time (maximum eclipse at about 4.24).



FIG. 1.

From Moose Jaw, Sask., the following extract was received: Amateur stations from districts W1, W2, W3 and W4 were very distinct on 40 metres at 1310* August 31, while the 20-metre band was dead. It would appear by this condition that the eclipse had considerable effect on short-wave signals as conditions at this period were completely reversed. The above districts are never heard during daylight at this location, whereas 20-metre signals at this hour are usually good. No effect was noticed on the lower frequencies.

Districts W1, W2, W3, and W4 cover roughly the eastern third of United States.

Stations of the Marine Department reporting short-wave reception are:—

Cape Hopes Advance, SS. N. B. McLean, from Ottawa, 22-metre band.
Experimental Farm Ottawa, from Morocco, Italy and California.
Moose Jaw, Sask., from Eastern United States.

* 1310 local time is 2010 G.M.T.

The following is a summary of some observations taken at Rigaud, Quebec:—

A party from the Engineering Department of l'Ecole Polytechnique in Montreal, under the direction of Professor J. A. Villeneuve, set up a station in Rigaud. Their object was to measure the height of the ionizing layers, but owing probably to oscillograph and transmitter trouble, their results on height measurements are inconclusive. They did, however, report that at the time of the optical eclipse they noted a sudden and rapid increase in received signal strength from stations a thousand miles away. This increase was noted at various wave-lengths between 20 and 80 metres.

From the evidence of short-wave reception it would appear that night-time or a partial return to night-time conditions prevailed during the optical eclipse, but there seems to be no indication in the reports of any effect occurring about the time of the predicted corpuscular eclipse. Reflection of these wave-lengths occurs in the F region, so any increase in strength may be a decrease in ionization and consequent decrease in absorption of the signal in the E or lower ionized region.

Conclusions

From the above observations it seems unlikely that there was any effect that could be ascribed to the predicted corpuscular eclipse, but it must be noted that many of the stations were not suitably situated for the observation of such effects. Cape Hopes Advance and McLean are probably the best situated with regard to place, but owing to their high latitude and the consequent low elevation of the sun they are not much better.

At the time of the optical eclipse more pronounced effects were found, the best example being the reception of Bowmanville at Yamachiche, by the Canadian Marconi Company, in which the transmission was directly across the path of optical totality. Although the trans-Atlantic transmissions are somewhat conflicting no great importance is attached to the decrease in signal strength at about 1900 G.M.T. of signals coming from GBB and GOK (see report of the Marconi Company). The Marconi Company seemed to consider that it was probably an aftermath of magnetic storms recorded prior to the eclipse.

All the above work gives corroborative evidence of the results in the two previous papers (1, 2), namely, that the ionization definitely decreases at the time of optical totality, and all results seem to support our conclusions that there was no corpuscular eclipse within the territory of our observations.

Acknowledgments

We wish to thank the Canadian Marconi Company, The Northern Electric Company, l'Ecole Polytechnique, and the Marine Department, for kindly giving us the results of their measurements.

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THE WIND TUNNEL DEVELOPMENT OF A PROPOSED EXTERNAL FORM FOR STEAM LOCOMOTIVES¹

By J. J. GREEN²

Abstract

The existing shape and arrangement of steam locomotives are such that smoke from the stack tends to sweep back along the boiler top and descend in front of the cab windows, seriously impairing forward vision. For the maximum degree of safety it is essential that the view from the cab, especially in a forward direction, should be unobstructed at all times. It is therefore desirable that some means be found for improving the manner in which the smoke is carried away from the stack. In addition to preventing the descent of smoke at the cab it is desirable that the external shape of the locomotive should be so modified as to result in a decreased air resistance, in view of the growing demand for economical running at increasingly higher speeds.

The paper describes work done in the wind tunnel of the National Research Laboratories and discusses the steps whereby an improved external shape has been evolved for locomotives such that the smoke is lifted over the cab thus making possible an unimpaired vision ahead.

The new design is the result of the application of elementary aerodynamics to the problem and aims at providing smoother air flow about the locomotive. Further, a layer of clean air is introduced between the smoke and the body of the locomotive and is responsible for maintaining the smoke above the cab. By removing the violent eddying flow about the locomotive the air resistance of the engine and tender has been reduced to the extent of some 35%.

Introduction

It is an unfortunate feature of the steam locomotive in its present state that the exhaust steam and smoke have to be emitted at a position some distance ahead of the cab. In recent times sporadic and inadequate attempts have been made to improve conditions for the engineer. With the development of higher-powered locomotives, the shorter stacks have increased the problem of lifting the smoke clear of the cab. When the locomotive is drifting, the absence of the exhaust steam jet in the stack allows the smoke to pour down over the top and sides of the boiler, seriously impairing vision from the cab windows (Figs. 8 and 9). This trouble is serious and may lead to accidents when the locomotive is approaching a station. If it is possible to lift the smoke well clear of the cab, it is quite likely that it will remain above the train so that the quantity of smoke and cinders entering the coaches will be reduced. No recent scheme for curing this trouble has been sufficiently successful to merit general adoption. A number of odd-shaped devices have been attached to the front of the boiler and the stack, but their lack of success is due to the fact that the aerodynamics of the entire locomotive is involved, particularly the front and upper surface of the boiler. Marshall (3) believes that the smoke trouble is caused by the "hollow space over the tender". This may be so in British practice, but the argument fails for Canadian and American locomotives where the "hollow space" is absent. The work done by Chapelon (1) at St. Cyr Aerodynamic Institute in 1928 is noteworthy.

¹ Manuscript received October 28, 1932.

² Contribution from the National Research Laboratories, Ottawa, Canada.

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A $\frac{1}{8}$ -scale model "Pacific" type locomotive was tested for the Orleans Company. Moving pictures were taken of the air and smoke flow as indicated by woollen streamers and magnesium oxide powder and the effect on smoke removal of various attachments was studied. Chapelon inferred from his work that devices placed round the stack were useless, but that lateral guard-screens at the front end of the boiler were effective in transforming the eddying flow about the locomotive into streamline flow. (Compare the effect of the Townend ring cowling (6) on air-cooled radial aero engines.) The Canadian National Railways and a number of other roads have experimented with deflectors at the stack, but they have not given satisfaction, as Chapelon predicted. Side guards have been used in England, but no information appears available as to their utility.

In the work which follows, it was hoped that radical changes in the entire locomotive shape would yield considerable improvement in smoke flow and resistance. It was felt that the use of side guards was an attempt to improve slightly what is already a very poor aerodynamic shape. Wind tunnel tests (Fig. 10) substantiate the statement that the entire upper surface of a modern locomotive is shrouded in eddies due to poor aerodynamic design. These eddies behind the stack, dome and valves trap the smoke which is then comparatively slowly dissipated since the eddies reduce considerably the average air velocity over the boiler. This retarding of the flow adjacent to the boiler is further enhanced by the crude shape of the boiler front. Removal of the smoke nuisance by changes in design which will give smooth high speed air flow over the boiler top and sides will obviously produce at the same time a reduction of the air resistance of the locomotive, and in recent times the reduction of running costs by minimizing air resistance has been slowly coming to the forefront. Work at the National Physical Laboratory for the L.M.S. and L.N.E.R. railways (2), and that of Tietjens (4, 5), may be cited as being typical of the attention being devoted to air resistance.

Air resistance at low or medium speeds is small in comparison with other sources of resistance and the locomotive itself has only about 30% of the total air resistance of the train. Since, however, the air resistance increases as the square of the speed, whereas other resistances rise only as some power of the speed less than unity, it is evident that, for the high speed passenger services nowadays common to railroad practice, air-resistance reduction merits attention in the quest for economy.

Nature and Scope of the Tests

In 1931 the Canadian National Railways requested the National Research Council to undertake an investigation of the above-mentioned problems with a view to improving their high speed locomotive design. A $\frac{1}{8}$ -scale model of the 6100 class engine and tender (Fig. 2), complete in every detail and constructed mainly of steel, was forwarded for use in the wind tunnel tests. The open tunnel with a jet 9 ft. in diameter, 13 ft. long and a maximum speed of 160 m.p.h. was eminently suitable for the work to be done.

A number of restrictions were imposed on the work at the outset; for example, owing to legal restrictions, certain features such as a bell, whistle, stairways at front and runways at sides cannot be eliminated, although their disposition might be changed. Further restrictions arose from the fact that the same general shape, sizes and clearances and operating arrangements were to be retained, and that definite accessibility for connecting rods, valve motion, axles and axle boxes was required. Despite the great saving in air resistance, no cowling could be tolerated over this mechanism, and the fire-box had to be left free to prevent blocking of the air-opening into the ash pan. Other structural limitations were imposed by the restricted position and height above the water level in the boiler of the feed-water heater. Clearance for the water supply pipes also limited the level of the top of the water tank. Finally all modifications were to be such as could be done economically and easily, with the bare minimum of alteration to the locomotive itself, and this practical aspect has been kept in mind throughout the work.

In view of the difficulty of extensive alteration to the scale model supplied, without damaging it, a wooden model was made to the same dimensions, reproducing all the main essentials, without the minor details (Figs. 2, 4 and 5). Both models were tested for resistance, and the ground effect on the steel model was measured both with a dummy ground and by utilizing the wooden model as a mirror image (Fig. 2).

The wind speeds employed in the tests ranged up to 170 ft. per sec., and above speeds of 30 or 40 ft. per sec. no scale effect existed, that is, the resistance coefficient obtained by dividing the resistance by the square of the wind speed is a constant within the limits of practical measurement. This linear variation of resistance with the square of the speed allows prediction of the full-scale locomotive resistance with greater assurance of accuracy.

Resistance Measurement

The method initially employed for measuring resistance was to suspend the model by wires and observe its "swayback" in the wind, computing the resistance from the observations. The drawbacks to this method were that the model oscillated considerably rendering observation difficult, the computations were laborious, and in the case of the wooden model it was necessary to load it with lead weights to limit the "swayback", which increased the number of wires in the jet and complicated the oscillation of the model.

In view of these objections, a rough subframe drag balance was built above the jet. The force on the model was transmitted by suspension and bracing wires to the subframe and thence to the scale pan of an accurate balance. Ground effect was supplied by a dummy ground (Fig. 4) supported beneath the model, just clear of the wheels.

Air Flow Around the Model

The velocity vectors of air currents around the model, with its various modifications, were studied by means of silk streamers attached to a grid

of very fine wire suspended just above the locomotive, and another just in front of the model. Additional threads were attached along the boiler sides and in front of the cab window. These threads proved very sensitive to changes in shape of the locomotive and were satisfactory in indicating the beneficial effect or otherwise of any specific modification, and in addition they were easily photographed for purposes of recording the air currents existing around the body of the model (Figs. 10 and 20).

All tests were done at zero yaw since the effect of side wind on a high speed locomotive is considered to be small, in so far as air resistance is concerned, and the results of the work were to be applied to high speed runs at maximum speeds in the neighborhood of 80 m.p.h. Very little room exists for improving the aerodynamic characteristics of railway coaches and since the air is excessively turbulent by the time the last coach is reached, it is doubtful if a special streamlined observation car at the rear end would be worthy of consideration. For these reasons and also because of the predominating importance of the smoke problem, no work was done on reducing train resistance.

The silk threads indicated the exceptionally poor airflow round the existing type of locomotive (Fig. 10) and showed how the general flow is deflected downwards all along the boiler sides. The existing shape of cab causes the air to pour down in front of the cab windows, and leaves no doubt as to the likelihood of their being obscured when the locomotive is drifting in the manner shown in Figs. 8 and 9.

General Outline of Results

By progressive steps there has been developed for the locomotive an improved shape which operates primarily by inducing a layer of pure air to enter under the smoke layer, such that a space enveloping the boiler and extending well above the top of the boiler is fed continuously with air uncontaminated by smoke. The construction of the model is such that this smoke-free layer is retained beneath the smoke with a minimum of intermingling of the two. Smooth entry for this clean air is effected by a new design for the locomotive front end. At the position of the cab further quantities of smoke-free air are induced upward in front of the cab windows, to augment the layer of pure air immediately above the locomotive. Here originally existed a downward current of smoke which necessitated the provision of a grating on the running board in front of the cab window, to allow for the disposal of the cinders collecting there.

The modifications to effect this change of flow result in a reduction of approximately 35% of the air resistance of the locomotive. The best model tested reduced the air resistance by 43%, but the cowling employed interfered with the accessibility required for the working parts.

Description of Tests and Results

Preliminary measurements of wind resistance were made with the $\frac{1}{4}$ -scale model. It was suspended at the centre of the wind tunnel jet by means of

eight wires (four 12-gauge wires to the locomotive, and four 16-gauge wires to the tender) attached at their lower ends to steel stirrups passing under the boiler and water tank of the model. The upper ends of the wires were fastened to steel I-beams on the platform above the jet. The wires were all identical in length and were arranged to be vertical. The ends of the wires at the model as well as at their upper attachments were pin-jointed. A steel scale graduated in hundredths of an inch was fastened horizontally on the side of the boiler as shown in Fig. 2. This steel scale was observed through a telescope set up at the side of the jet, and for each wind speed employed the "swayback" of the model was observed through the telescope. The method used for computing wind resistance can be followed by reference to Fig. 1.

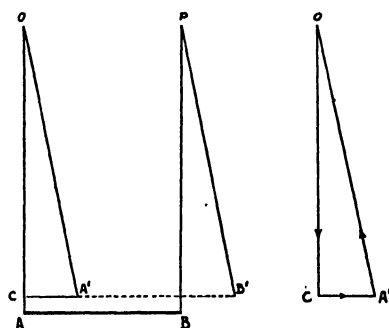


FIG. 1.

Let AB represent the model suspended by parallel wires of equal length, OA , PB , in a wind whose direction is indicated by the arrow. Suppose the model sways back to a position $A'B'$. Equilibrium of the system is determined by the weight of the model, the air resistance or drag, and the tension in the supporting wires. $OA'C$ is the triangle of forces where the length OC is proportional to the total model weight W , and the length $A'C$ is proportional to the resistance R . ($OA = OA' = PB$ = length of supporting wires measured from pin

joints at each end.)

Let $D = A'C$ the amount by which the model has swayed back.

$$\text{Then } OC = \sqrt{(OA')^2 - D^2} = \sqrt{(OA' + D)(OA' - D)}.$$

$$\text{Now } \frac{R}{W} = \frac{A'C}{OC} \text{ or } R = \left(\frac{D}{OC}\right) \times W = \frac{D}{\sqrt{(OA + D)(OA - D)}} \times W.$$

Knowing W and OA and measuring D , it is possible to calculate R , the wind resistance.

Table I gives the results for the metal model for which the total weight, W , was 318.75 lb. The length of suspension wires was 11 ft. 11 $\frac{3}{8}$ in.

The resistance coefficient in the last column includes the air drag of the supporting wires. The existence of scale effect is seen at speeds below about 40 ft. per sec. where the value of $\frac{R}{V^2}$ changes with V . Above this speed the value of the resistance coefficient is reasonably constant within the limits of experimental accuracy.

Assuming then that the resistance coefficient $\frac{R}{V^2} \times 100$ is the same for full scale as at the higher speeds in the model tests, the full-scale resistance at

any wind speed V ft. per sec. is derived by multiplying the value of $\frac{R}{V^2}$ by V^2 $\times \left\{ \frac{1}{\text{model scale}} \right\}^2$, so that for a $\frac{1}{12}$ -scale model the multiplying factor will be $(V^2 \times 144)$, the relative resistances of model and full scale being in the ratio of their frontal areas.

TABLE I
RESULTS OBTAINED WITH THE METAL MODEL

Wind speed, V , ft./sec.	Amount of swayback, D , in.	$\sqrt{(OA + D)(OA - D)}$	Wind resistance, R , lb.	Coefficient $\frac{R}{V^2} \times 100$
20	0.24	143.375	0.534	0.133
30	0.55	143.374	1.223	.136
40	1.00	143.372	2.223	.139
50	1.56	143.366	3.468	.139
60	2.28	143.357	5.070	.141
70.4	3.12	143.341	6.938	.140
79.8	4.01	143.319	8.918	.140
90	5.14	143.283	11.434	.141
100	6.38	143.233	14.198	.142
110.5	7.77	143.164	17.300	.142
120	9.14	143.084	20.361	.141
154	15.12	142.575	33.750	.142
170.2	18.08	142.230	40.455	.140

Correction of Results for Drag of Supporting Wires

The reaction at the locomotive due to the wire-suspension drag is given as a contribution to the total resistance measured in the tests, and must therefore be deducted in order to evaluate the resistance of the locomotive and tender alone. Since the curve relating to the resistance of wires, their diameters and wind speed is well known, the value of the resistance of those lengths of the suspension wires included by the air jet was easily calculated for each wind speed used in the tests, and the necessary correction applied.

This gave a mean value of $\frac{R}{V^2} \times 100 = 0.1135$ for the model alone over the the range of speeds for which the coefficient is constant, R being in lb. and V in ft. per sec.

Ground Effect

The resistance measurements on the metal model were not as yet applicable to actual practice in view of the fact that the model had been freely suspended, and the presence of the ground in full scale had not been allowed for.

Two methods suggested themselves for introducing ground effect. It is well known that the effect of a boundary surface on the air flow past a body in its neighborhood is the same as if the boundary was replaced by a second model so placed with respect to the first as to constitute a "mirror image"

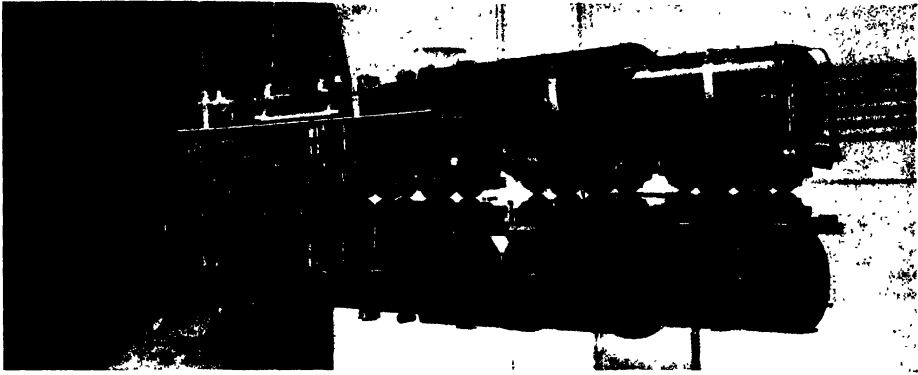


FIG. 2. *Model of C.N.R. 6100 class locomotive and tender ($\frac{1}{12}$ -scale) suspended in wind tunnel jet with wooden dummy to give ground effect.*



FIG. 4. *Wooden model with dummy ground suspended in wind tunnel.*



FIG. 10. *Air flow over unmodified model as indicated by silk threads. Wind speed, 45 m.p.h.*



FIG. 20. *Air flow over modified model as indicated by silk threads. Wind speed, 45 m.p.h.*

of the first model in the boundary. The wooden model was then used in the first method for determining ground effect. Fig. 2 shows the two models under test. Knowing the resistance of the metal model freely suspended and subsequently measuring the resistance of the wooden model freely suspended, the sum of these two resistances exceeds the resistance of the combined pair by an amount which represented the effect of the ground. Assuming this effect to work equally on both models, half the effect gives the individual ground interference on any one model.

It was found that the ground effect was such as to reduce the resistance of the locomotive and this becomes obvious when it is considered that the action of the ground is such as to retard the air passing underneath the model.

The second method of evaluating ground effect was to suspend a dummy ground underneath the model, just clear of the wheels, and to measure the resistance of the model with this ground in place. Such a method produces results which are directly applicable to the case of a full-scale locomotive running on the tracks, and by evaluating the difference between these results and those for the freely suspended model, a second estimate of the ground effect could be made and compared with that derived by the use of a "mirror image".

Results

Speed range 0 to 175 ft. per sec.

Mean coefficient of resistance for metal model freely suspended,

$$\frac{R_s}{V^2} \times 100 = 0.1135.$$

Mean coefficient of resistance for wooden model freely suspended,

$$\frac{R_w}{V^2} \times 100 = 0.1062.$$

Mean coefficient of resistance for combined pair freely suspended,

$$\frac{R_s + R_w - 2d}{V^2} \times 100 = 0.2054,$$

where d is the interference effect on each model due to the ground. By

addition a set of values for $\frac{R_s + R_w}{V^2} \times 100$ over the speed range are calculated

and by subtracting the corresponding values of $\frac{R_s + R_w - 2d}{V^2}$ a set of values

for $\frac{2d}{V^2} \times 100$ are derived. The results gave a mean value for $\frac{d}{V^2} \times 100$ of 0.0067, as the interference effect on each model due to ground.

Correcting the resistances of the two models for ground effect, the resistance of the wooden model, including ground effect, is given by

$$\frac{R}{V^2} \times 100 = 0.0995,$$

the resistance of the metal model, including ground effect, is given by

$$\frac{R}{V^2} \times 100 = 0.1068,$$

R in lb. and V in ft. per sec.

These values are obtained by subtracting the ground effect,

$$\frac{d}{V^2} \times 100 = 0.0067,$$

from the resistance coefficient for each model freely suspended.

The full-scale locomotive resistance at V m.p.h. is then given from the results with the steel model as

$$R = \frac{0.1068}{100} \times \frac{88^2}{60^2} \times 144 V^2 \text{ lb.},$$

or

$$R = 0.3308 V^2 \text{ lb.}$$

Measurements with a Subframe Drag Balance

Fig. 3 shows the general arrangement of this drag balance. The subframe is constructed of angle iron welded together, and is suspended from two vertical frames which will be designated by the terms upstream and downstream.

The downstream frame supports the subframe on two knife edges, whereas the upstream frame supports the subframe on a single cone pivot. At its upper end, the downstream vertical frame is suspended from two knife edges, the V-blocks being fixed on the upper flanges of two longitudinal I-beams resting on the balance platform. The upstream vertical frame is likewise pivoted on two knife edges located on the upper flanges of the I-beams. This upstream frame is also fitted with sensitivity weights at its upper end, and is arranged to transmit the horizontal drag force as a vertical force acting on the scale pan of a Dayton balance. The four upper knife edges from which the two vertical frames are suspended are all in one plane. Similarly the two knife edges and cone pivot on which the subframe rests are all in another plane.

The horizontal spacing of these points of support is the same in both planes and the vertical spacing is the same at the upstream and downstream frames. By virtue of this the subframe remains horizontal as the balance swings, and the two vertical frames remain parallel as they swing out of the vertical. The balance includes a dash pot for damping oscillations.

The ratio of the moment arms is such that the balance indicates 1.83 times the drag force on the model.

The first test made with the subframe drag balance was to repeat the measurement of the resistance of the wooden model freely suspended with no ground effect included, the idea being to check the agreement between the subframe balance measurements and those made by observing swayback.

After correcting for wire drag, the resistance, R lb., of the freely suspended wooden model was found to be given by $\frac{R}{V^2} \times 100 = 0.1068$, compared with 0.1062, the value deduced from swayback measurements.

The wooden model was initially suspended from the subframe by four 12-gauge wires and four 16-gauge wires and by removing these in pairs, making resistance measurements after each removal until only two wires of each gauge remained, it was possible to deduce an experimental value for the

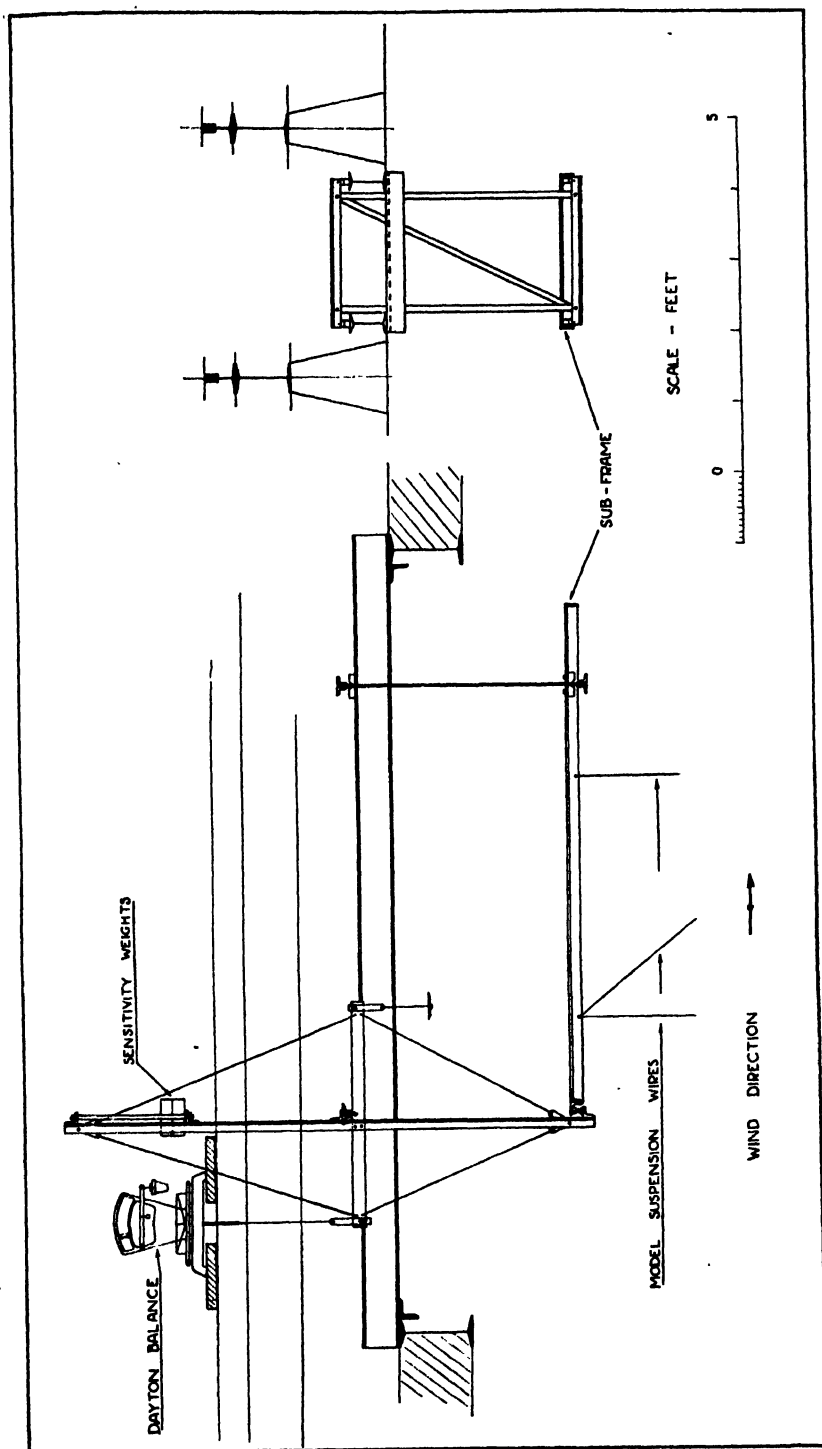


FIG. 3. Diagram of the subframe drag balance.

wire-drag contributions. Since these included the turnbuckles incorporated in the wire suspension system, in correcting the readings for the drag of the remaining wires it was preferable to use these experimental corrections rather than the theoretical corrections used heretofore.

Use of a Dummy Ground

Fig. 4 shows the wooden model suspended from the subframe balance by four 16-gauge wires, and braced by two inclined wires of very small diameter. The dummy ground is shown in place; it was made to be over twice as wide as, and considerably longer than, the model, and was braced rigidly by wires attached as shown in Fig. 4. It was provided in side elevation with a rounded nose and a tapering tail, and was rectangular in plan form. This dummy ground was used throughout the remaining work.

Results

The mean value over the speed range 0 to 130 ft. per sec. of the resistance R lb. of the wooden model with dummy ground present was found to be given by

$$\frac{R}{V^2} \times 100 = 0.1089,$$

where due correction has been made for the drag of the vertical suspension wires, but no allowance has been made for the drag of the inclined bracing wires.

The mean value of the resistance of the model, with no ground present, over the same speed range and uncorrected for the inclined wire drag was found to be

$$\frac{R}{V^2} \times 100 = 0.1149.$$

The mean value of the ground effect over the same speed range, obtained by averaging the differences between the two sets of readings whose mean values are given above, was found to be

$$\frac{d}{V^2} \times 100 = 0.0066,$$

which agrees very closely with the value of 0.0067 found by the previous method for determining ground effect, in which a mirror image of the model was used.

Modifications of the Model and their Effect on Resistance and Air Flow

The problem of modifying the external shape of the locomotive was simplified by the realization that improved air and smoke flow goes hand in hand with reduced air resistance. Features on existing locomotives which upset the air flow are the feed-water heater and number lights, the dome, valves and turrets, the poorly shaped front and the exposed valve motion and running gear. All these contribute to the resistance by producing eddies.

The first modifications were made with a view to reducing wind resistance by improving the airflow about the sides and top of the locomotive, and these will be considered in the order in which they were made and numbered accordingly. Fig. 5 shows the wooden model before modification. All details are not included in this drawing.

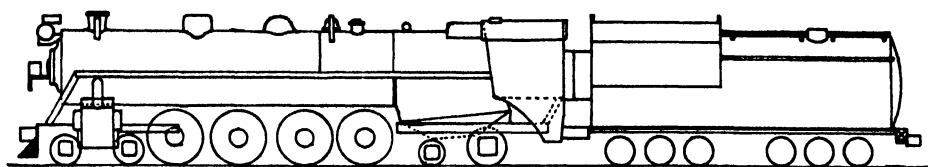
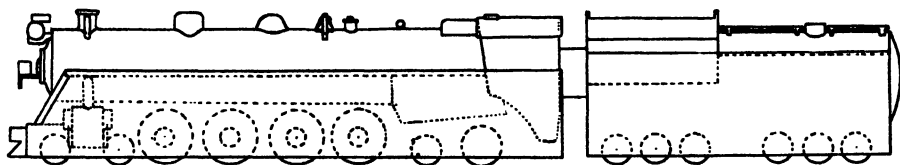


FIG. 5. *Drawing of original model unmodified.*

(1) *Side curtains on the locomotive.* In order to shield the cylinders, valve motion, running gear, etc., side curtains were fitted to the model below the running boards and extending down to the level of the bottom of the cowcatcher (see Fig. 6). These side curtains reached from the cowcatcher or pilot to the rear of the locomotive cab and were made of sheet metal. A solid wooden pilot was made to replace the normal type and a sloping sheet metal front extending from the top of the pilot to the bottom of the smoke box was incorporated. The reason for these changes was to prevent air from entering underneath the locomotive through the cowcatcher bars or just below the

1.
2.



3.

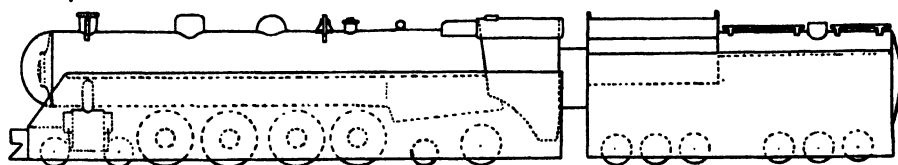


FIG. 6. *Drawings of model incorporating modifications 1, 2 and 3.*

boiler. By thus shielding the running gear both by the side curtains and by so closing the front below the boiler as to exclude air, it was hoped that wind resistance could be reduced and a smoother flow achieved for the air passing along the sides of the locomotive.

The wind speeds employed in this and subsequent tests were 20, 40, 60, 80, 100, 120 and 130 ft. per sec., but the average values only of the resistance

divided by the square of the wind speed have been presented here. Due correction has been made for the drag of the vertical suspension-wires but no allowance has been made for the relatively smaller drag of the two inclined bracing wires, owing to the difficulty of correcting for them. In view of their small effect, and since also the tests were designed to measure differences arising from modifications, in which case wire drag balances out completely, this correction was deemed unimportant.

Drag of original unmodified model R lb. at V ft. per sec. given by

$$\frac{R}{V^2} \times 100 = 0.1089.$$

Drag of model with side curtains on locomotive and solid pilot, etc., as outlined above given by

$$\frac{R}{V^2} \times 100 = 0.1088.$$

At first sight this result was disappointing but it was seen on further consideration that the effectiveness of any particular modification in reducing resistance would depend very considerably on the extent to which other changes had been already made to reduce resistance. It was argued that adding side curtains to an otherwise unchanged model would produce only a very small change in resistance, whereas their effect on a model which had been carefully streamlined elsewhere would be very considerable indeed. This argument was completely vindicated by subsequent tests.

(2) *Side curtains on the tender.* Leaving the model already modified as above, sheet metal side curtains were added to the tender extending from runways to the same lower level as those on the locomotive and reaching from front to rear of the tender (see Fig. 6). Their object was to close in the curved sides of the water tank and the frame and axle boxes of the tender.

Drag of the model so modified was given by

$$\frac{R}{V^2} \times 100 = 0.1036.$$

This indicates a greater saving than that effected by the side curtains on the locomotive. This saving, it is argued, would not be so appreciable had the side curtains been absent from the locomotive and would have been greater still had the rest of the model been streamlined.

(3) *Modifications to the boiler front.* The feed-water heater, headlamp and side staircases were removed and an approximately hemispherical front was fitted to the smoke box, otherwise the model was as modified in (1) and (2). A diagram of the model thus modified is given in Fig. 6.

The changes were intended to provide smooth flow over the locomotive front and to remove obstructions likely to disturb this flow.

Drag of model given by

$$\frac{R}{V^2} \times 100 = 0.0763.$$

This represents some 30% saving in resistance on that of the original model. The feed-water heater was suspected of causing considerable disturbance to smooth flow and its removal undoubtedly accounts for part of the reduction

in drag. The curved nose on the front of the smoke box ensures smooth flow initially which offsets the disadvantages of bad shape met with subsequently. It is well known that changes to the front of a body have considerable effect on the flow around it and, although this is usually applied to the treatment of streamlined bodies, it appears in some measure to be true of such a poor aerodynamic shape as a locomotive.

(4) *Modifications to the top of the boiler.* With the model as already modified, the following changes were made.

The whistle and bell were removed and a metal cowl flush with the centre of the cab roof and having the same roof contour but vertical sides was fitted over the turrets, valves and dome, extending forward to the centre of the sand dome, as shown in Fig. 7.

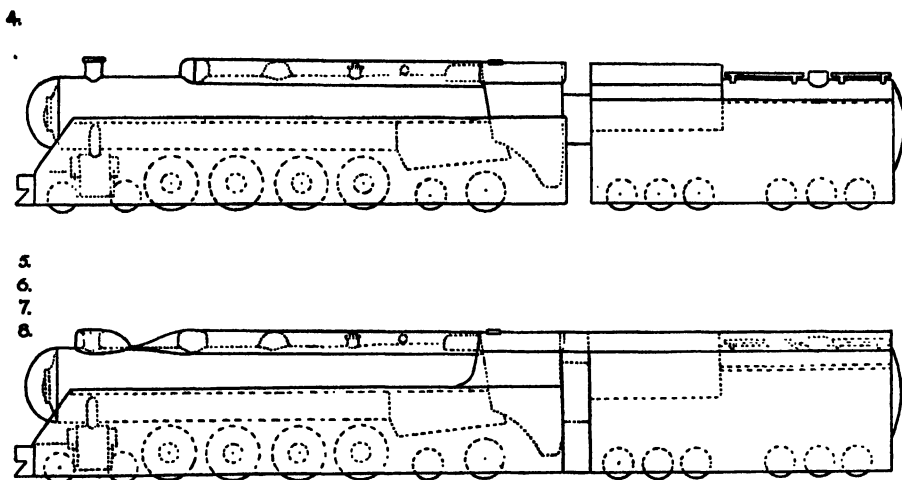


FIG. 7. Drawings of model incorporating further modifications 4, and 5, 6, 7, 8.

These changes were intended to remove the eddying flow over the upper surface of the boiler due to obstructions presented by the various items located on the boiler top.

Drag of the modified model given by

$$\frac{R}{V^2} \times 100 = 0.0710.$$

This modification was expected to be very beneficial as regards smoke removal by introducing smooth flow conditions behind the stack, the reduction in resistance being evidence of the removal of the eddying flow.

(5) *Modifications to the stack region.* The front of the metal cowl over the turrets, valves and dome was sloped down in a gentle curve to the top of the boiler just behind the stack and a streamlined tail was added to the stack (see Figs. 7 and 23). This latter modification was intended to reduce wind resistance as well as providing what is important from the point of view of smoke removal, *vis.*, smooth flow round the stack and an absence of an eddying region behind the stack.

Drag of the model is given by

$$\frac{R}{V^2} \times 100 = 0.0698,$$

a saving in resistance being effected as anticipated.

(6) *Modifications to the tender.* A cowling conforming to the cab roof was fitted over the entire tender, with the object of removing the space between the cab roof and the first coach roof caused by the lower level of the top of the water tank. This cowling is depicted in Fig. 7.

Drag of the model is given by

$$\frac{R}{V^2} \times 100 = 0.0666,$$

a reduction in resistance resulting from removal of the depression behind the coal bunker where eddying of the flow was occurring.

(7) *Cab modifications.* The gap between the cab and tender was closed by a cowling flush with the sides and roof of the cab as shown in Fig. 7.

Drag of the model given by

$$\frac{R}{V^2} \times 100 = 0.0666.$$

Closing in this gap had absolutely no effect on resistance. In view of this fact it appears useless to attempt to reduce train resistance by closing the gap between coaches, and more so, since the air is more turbulent by the time it reaches the coaches.

(8) *Cab modifications.* As already indicated the slope of the cab front is such as to create a down draught of air at the cab window. To avoid this the cab front was filled in to slope backwards from the running board and the overhang of the cab roof was eliminated. Generous fillets and curves were employed to eliminate corners and sharp edges, and a generous smooth curve between running board and cab front was considered necessary. This modification is shown in the lower drawing of Fig. 7.

It was intended that air taken from the sides of the boiler above the running boards should be shot upwards in front of the cab windows to keep them clear.

Drag of the model was found to be given by

$$\frac{R}{V^2} \times 100 = 0.0620,$$

indicating that a considerable reduction in resistance results from these simple modifications to the cab.

The combined effect of all the foregoing modifications was thus found to produce a 43% reduction in the air resistance of the original model.

Having gone as far as possible in removing the smoke trouble by improving the design of the locomotive from an aerodynamic standpoint, it remained to examine the flow of air over the original model and over the modified model, in order to see how successful this improvement was as regards smoke removal, and to add any special devices or modifications in order to increase the effectiveness of the new shape in keeping the smoke from the cab windows.

Investigation and Improvement of the Smoke Flow

For the purpose of examining the flow around the model a number of methods were considered before the final decision to use silk streamers was made. The use of titanium tetrachloride for exhibiting the flow and allowing photographs to be made would have been ideal but for the fact that the corrosive vapor formed by it could not be tolerated. The use of metal sheets painted with kerosene and lampblack placed round the model



FIG. 8. C.N.R. 6100 locomotive drifting, showing poor flow of smoke.

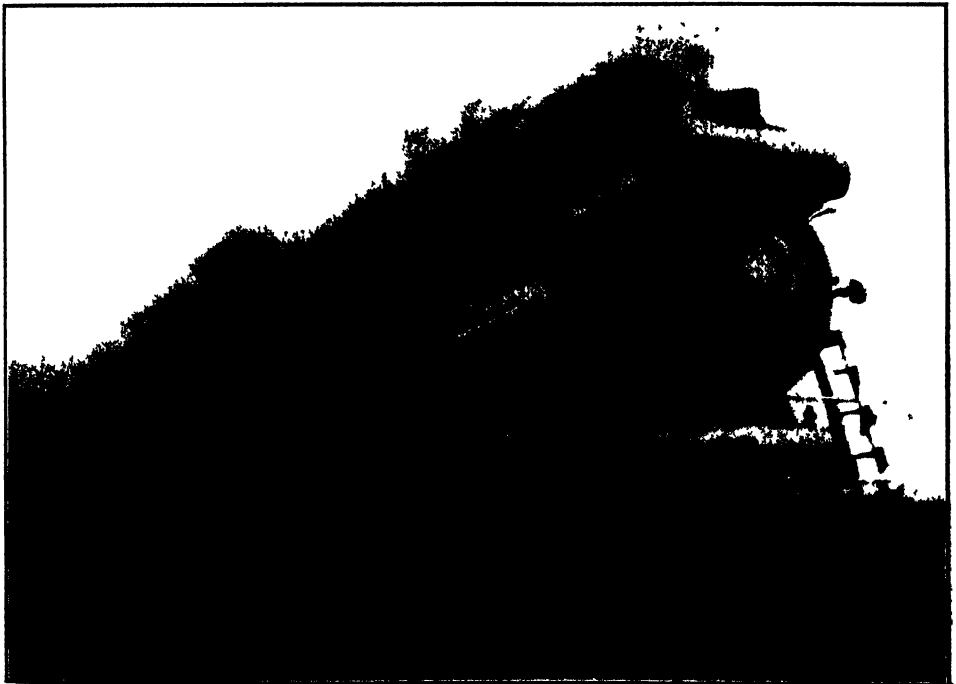


FIG. 9. C.N.R. 6100 locomotive drifting, showing cab window obscured by smoke.

in the plane in which the flow is to be investigated was considered but not employed, owing to the inevitable distortion of the flow by the metal sheet coupled with the fact that only the flow adjacent to the metal sheet is exhibited.

In Figs. 8 and 9 the flow of smoke from the stack of a C.N.R. 6100 engine is shown when the locomotive is drifting. It can be seen from these photographs how the smoke clings to the top of the boiler and blows down round the cab window.

Fig. 10 is a photograph of the original wooden model set up in the wind tunnel with silk streamers attached. The wind speed was 45 m.p.h. and the directions of the air currents around the model are indicated by the disposition of the silk threads.

The following deductions are clearly indicated by the photograph:—

(a) The large eddy behind the smoke stack, caused partly by the feed-water heater, traps the smoke from the stack in the manner shown by the streamer attached to the top of the stack.

(b) A region of eddying flow occurs behind the dome, turrets, etc., on top of the boiler, shown by the streamers attached to these portions of the locomotive.

(c) Considerable downward flow occurs along the boiler sides, exhibited by the streamers attached to the side of the boiler at the dome and at the bell.

(d) A considerable down draught of air occurs at the cab window, shown up clearly by the two threads attached to the boiler side at this position.

In Fig. 11 a drawing has been made of the air flow around the partially modified model as exhibited by the use of silk streamers. The following important points are indicated:—

(a) There is no eddy behind the stack; the thread from the top of the stack trails out behind with a slight upward trend.

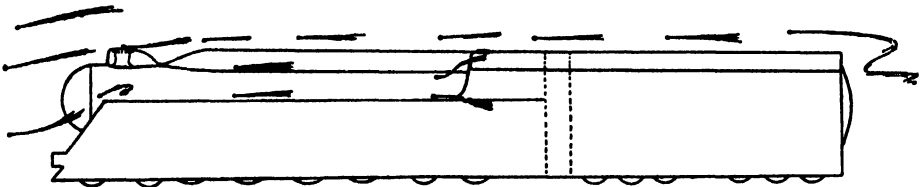


FIG. 11. *Type of flow over model as modified in lower drawing of Fig. 7, exhibited by silk threads.*

(b) Steady smooth flow conditions exist over the top of the boiler.

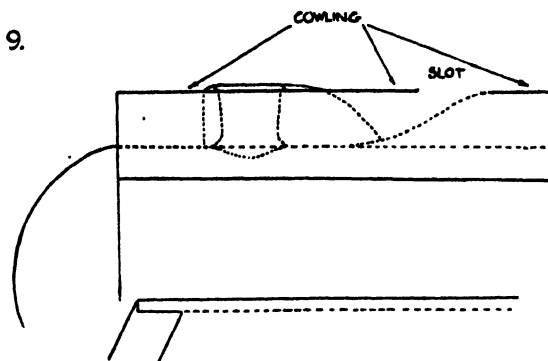
(c) There is no downward flow along the boiler sides.

(d) The flow over the cab window is mainly upwards although slight downward flow occurs where the cab front meets the running board, and a slight tendency exists for the air to spill round the sides of the cab.

It is obvious that the flow over this model represents a considerable improvement over the original type of flow, although room still remains for improvement at the cab and at the stack if the smoke is to be positively lifted clear of the locomotive.

Special Modifications for Removing the Smoke

(9) *Changes to stack region.* In order to keep the smoke layer from blowing down into contact with the top surface of the locomotive, an attempt was made to collect a volume of pure air from in front of the stack and to discharge it behind the stack, underneath the smoke layer. The first attempt to do this was made by extending forward the cowl which covered the dome, turrets, etc., so that it reached past the smoke stack to the front of the boiler where it was left open to catch air. The smoke stack just protruded through the top of this cowl and a slot was cut in this new portion of the cowl where it butted on to the old cowl in order to allow exit for the air caught in front of the stack. These changes are shown in Fig. 12 (upper drawing).



10.

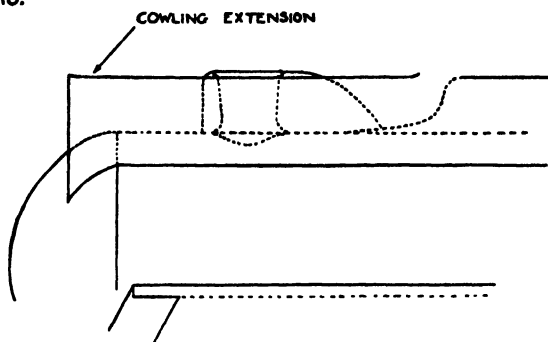


FIG. 12. Drawings of model incorporating further modifications 9 and 10.

This arrangement was only moderately successful. The smoke layer was indeed lifted by the introduction of a clean air layer between it and the locomotive, but not to the extent desired.

(10) In order to catch a greater volume of air the cowl was extended forward some two inches and its sides dropped down to the rounded nose of the boiler as shown in the lower diagram of Fig. 12. The two sides of the cowl were opened out to give a flared entrance. The rear surface of the slot which ran in a smooth surface from the top of the old cowl to the boiler top behind the stack was modified to give a greater upshoot to the air discharged from the slot.

These changes produced an improved flow. The streamers indicated the existence of a very strong ascending air current from the slot behind the stack.

(11) *Cab front modifications.* To prevent the spilling of air round the sides of the cab, side plates were fitted to the cab such that they protruded forward some short distance from the cab front. These side plates in conjunction with the boiler sides formed scoops in front of the cab windows (see Fig. 13), which functioned by shooting the air upwards over the windows. These side

plates improved the flow by eliminating both the slight down trend of the air near the running board and the spilling round the cab sides.

It was felt that the flow over this model was satisfactory and a series of measurements of its wind resistance were made at various speeds to see what effect these changes had made on drag.

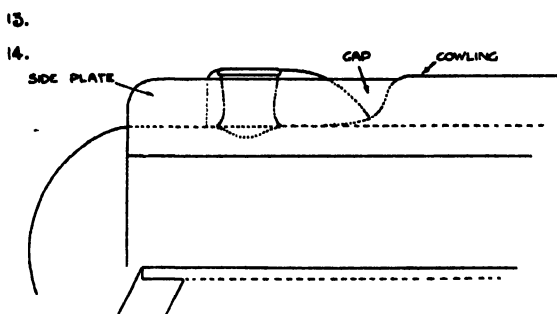
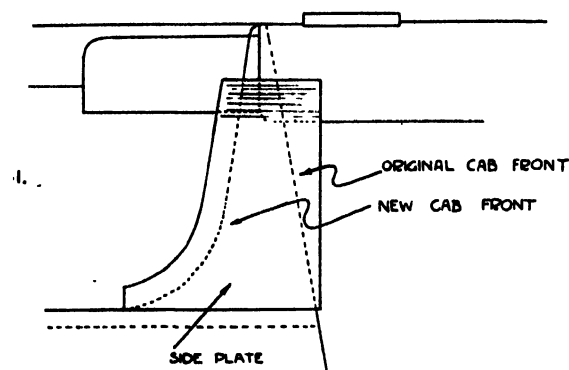


FIG. 13. Drawings of model incorporating further modifications 11, 13 and 14.

the stack, the model was modified by removing the roof of the cowling forward of the slot as in Fig. 13 (lower drawing). The idea here was to remove the interference of the roof over the passages on either side of the stack. It was felt that it merely retarded the pure air layer and contributed nothing to the scheme.

Drag of the model was then found to be given by

$$\frac{R}{V^2} \times 100 = 0.0665.$$

With these modifications the air flow was found to drop slightly just behind the stack before finally ascending over the cowling.

(14) The length of the gap between the streamlined tail of the stack and the curved surface leading to the top of the cowling was reduced in order to remove the sudden drop of the flow just behind the stack. This was successful. Fig. 13 (lower drawing) shows this modification.

(15) The front ends of the running boards were rounded off to remove the eddies formed by the sharp corners and side plates were added to act as

The results gave a mean value of

$$\frac{R}{V^2} \times 100 = 0.0727.$$

It is evident that the special modifications made to lift the smoke at the stack had considerably increased the air resistance of the model. It was assumed that the extension to the cowling had disturbed the flow over the round nose of the model and caused the sudden rise in resistance.

(12) Accordingly the model was changed by removing the 2-in. extension added in 10.

Drag of the model was then given by

$$\frac{R}{V^2} \times 100 = 0.0685.$$

(13) Leaving the sides of the extended cowling untouched on either side of

scoops, as shown on the left in Fig. 14. This improved the flow, lifting it all along the boiler sides, but the resistance was increased.

Drag of the model was given by

$$\frac{R}{V^2} \times 100 = 0.0798.$$

This large increase in drag was caused by the side plates giving rise to large eddies in the flow round the sides of the model.

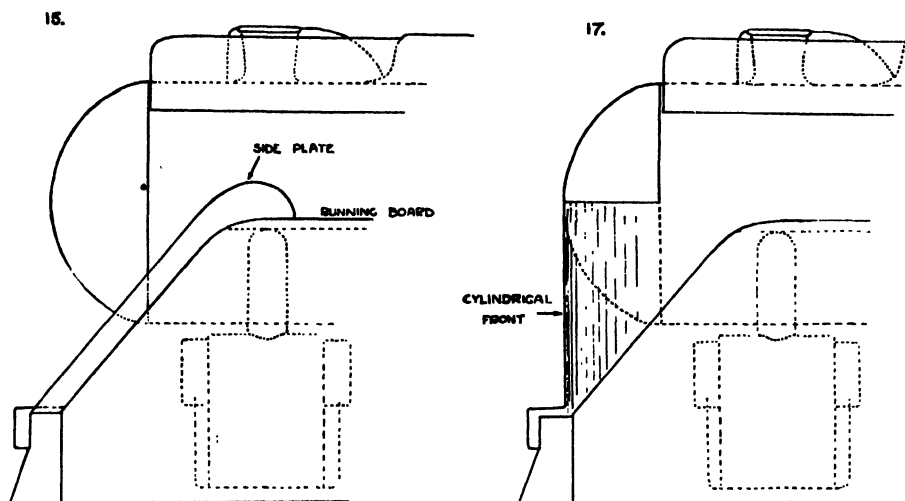


FIG. 14. Drawings of model incorporating further modifications 15 and 17.

(16) Removed side plates, running boards left rounded at their front ends. Drag given by

$$\frac{R}{V^2} \times 100 = 0.0654,$$

restoring the resistance to a figure approximately the same as that before the addition of the side plates at the front ends of the running boards.

(17) *Changes to the boiler front.* A cylindrical surface was added between the rounded nose of the boiler and the sloping front, as shown on the right in Fig. 14. The intention was to provide smooth flow conditions for the air entering the space above the running boards along the boiler side.

Drag given by

$$\frac{R}{V^2} \times 100 = 0.0647,$$

indicating a slight reduction in air resistance due to smoother flow conditions.

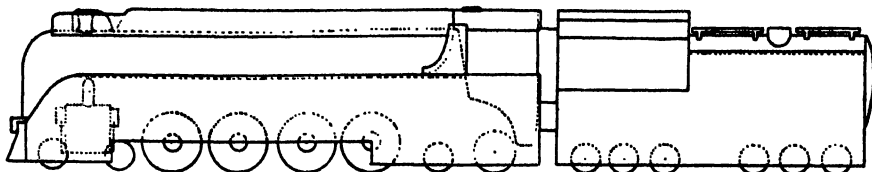
(18) *The tender.* In view of the operating restrictions cited in the introduction the tender could not be fitted with a cowling over the water tank, and runways had to be left at the sides of the coal bunker. A cowling conforming to the cab roof was left over the coal bunker.

Drag of model was then given by

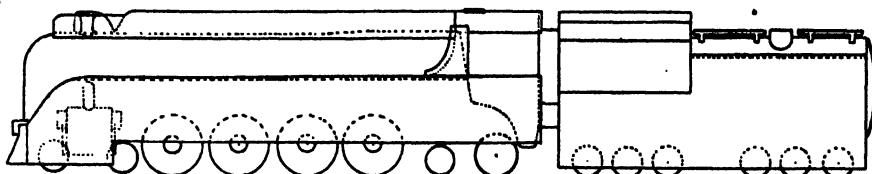
$$\frac{R}{V^2} \times 100 = 0.0633.$$

(19) *Side curtains.* Operating restrictions will not at present tolerate the complete closing in of valve motion, cylinders, running gear, axles, etc., and for this reason the side curtains had to be cut down in size to leave exposed the several places where inspection and servicing are required. To examine

19.



20.



21.

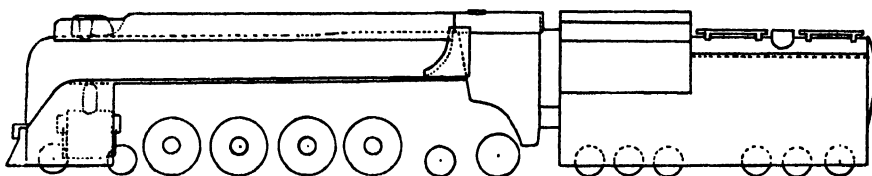


FIG. 15. Drawings of model incorporating further modifications 19, 20, 21.

the contribution of side curtains to the saving of resistance, this cutting down of their size was done in stages, the results being as follows:—

Full-size side curtains,

$$\frac{R}{V^2} \times 100 = 0.0633.$$

Side curtains cut off below the level of the centres of the driving wheels and from behind the cylinders to the centre of the rear driving wheels as shown in Fig. 15 (upper drawing),

$$\frac{R}{V^2} \times 100 = 0.0650.$$

(20) Side curtains cut away below level of driving wheel centres from the rear of the cylinders to the rear of the cab, as shown in middle drawing of Fig. 15,

$$\frac{R}{V^2} \times 100 = 0.0656.$$

(21) Side curtains removed completely from the rear of the cylinders to the rear of the cab as in lower drawing of Fig. 15,

$$\frac{R}{V^2} \times 100 = 0.0695.$$

(22) Side curtains cut away below the level of the top of the driving wheels from rear of cylinders to rear of cab, as in Fig. 16,

$$\frac{R}{V^2} \times 100 = 0.0702.$$

(23) Tender side curtains cut off below the level of the frame of the tender as shown in Fig. 16,

$$\frac{R}{V^2} \times 100 = 0.0715.$$

22.

23.

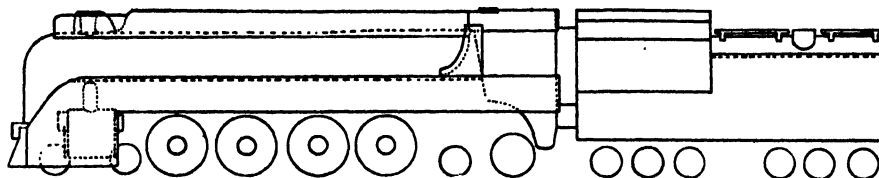


FIG. 16. Drawing of model incorporating further modifications 22 and 23.

(24) Side curtains completely removed from locomotive,

$$\frac{R}{V^2} \times 100 = 0.0846.$$

(25) Side curtains completely removed from locomotive and tender,

$$\frac{R}{V^2} \times 100 = 0.0865.$$

These results show how very vital side curtains are when the rest of the model is well streamlined, yet the addition of side curtains to the original otherwise unmodified model had practically no effect on resistance.

With present requirements regarding accessibility the arrangement shown in (22) was accepted as being possible to reproduce on full scale.

(26) *Final modifications to the front of the model.* In order to simplify the front of the locomotive and to induce the flow of large quantities of clean air from the front of the model over the top

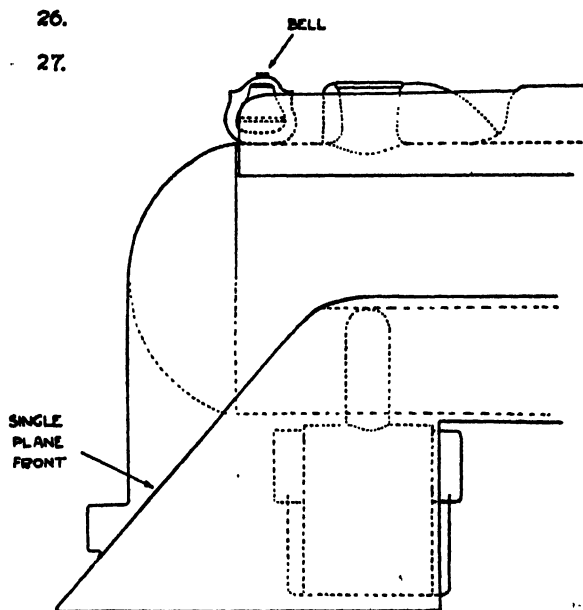


FIG. 17. Drawing of model incorporating further modifications 26 and 27.

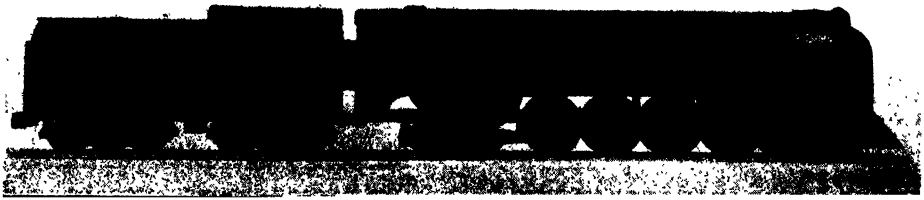


FIG. 18. *Model as finally modified, side view.*

and sides of the boiler, a sloping solid pilot running in one plane from as near the rail level as possible up to the running boards on either side of the boiler was fitted in place of the original one. This modification results in the style of front depicted in Figs. 18, 19 and the drawings of Fig. 17.

With this change effected and the model otherwise as in 23 above, the model drag was given by

$$\frac{R}{V^2} \times 100 = 0.0700.$$

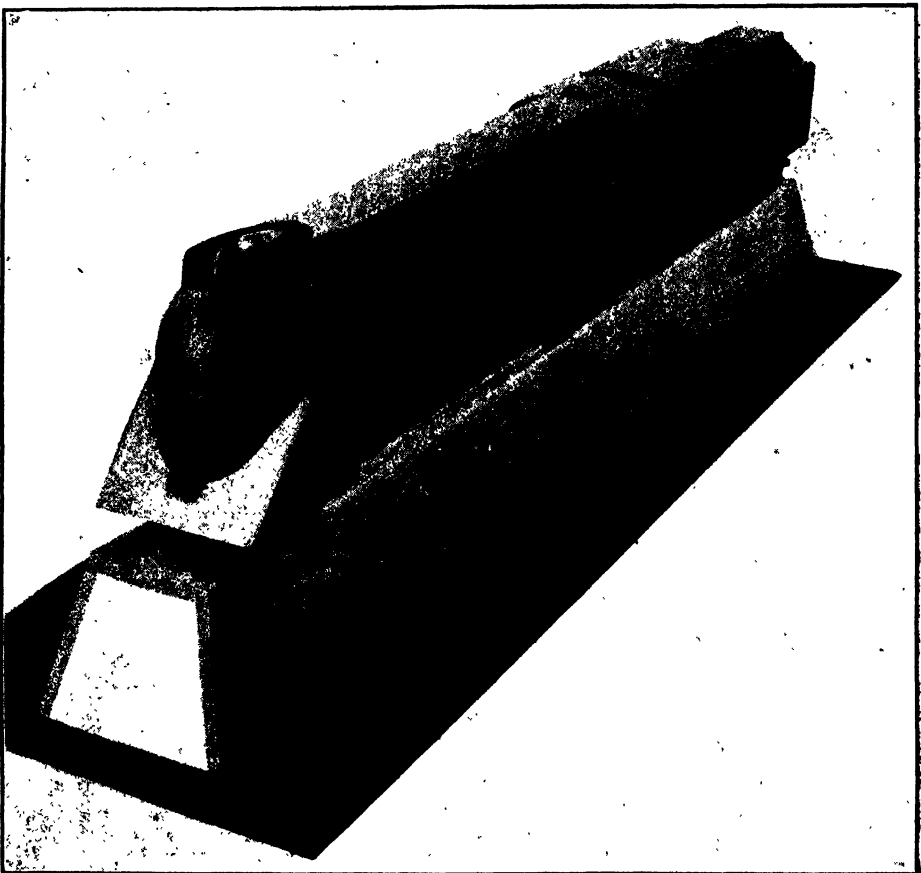


FIG. 19. *Model as finally modified, oblique view.*

(27) *Location of the bell.* The bell was originally placed on the side of the boiler but in this position it upsets the flow of air over the cab window. It was now placed in front of the stack (see Fig. 17) with its frame edgewise on to the wind and in this position it was found to have a shielding effect on the top of the stack, such that the flow in the region of the stack ascends much more rapidly than formerly, as shown in the photograph Fig. 20.

With the bell so located,

$$\frac{R}{V^2} \times 100 = 0.0706.$$

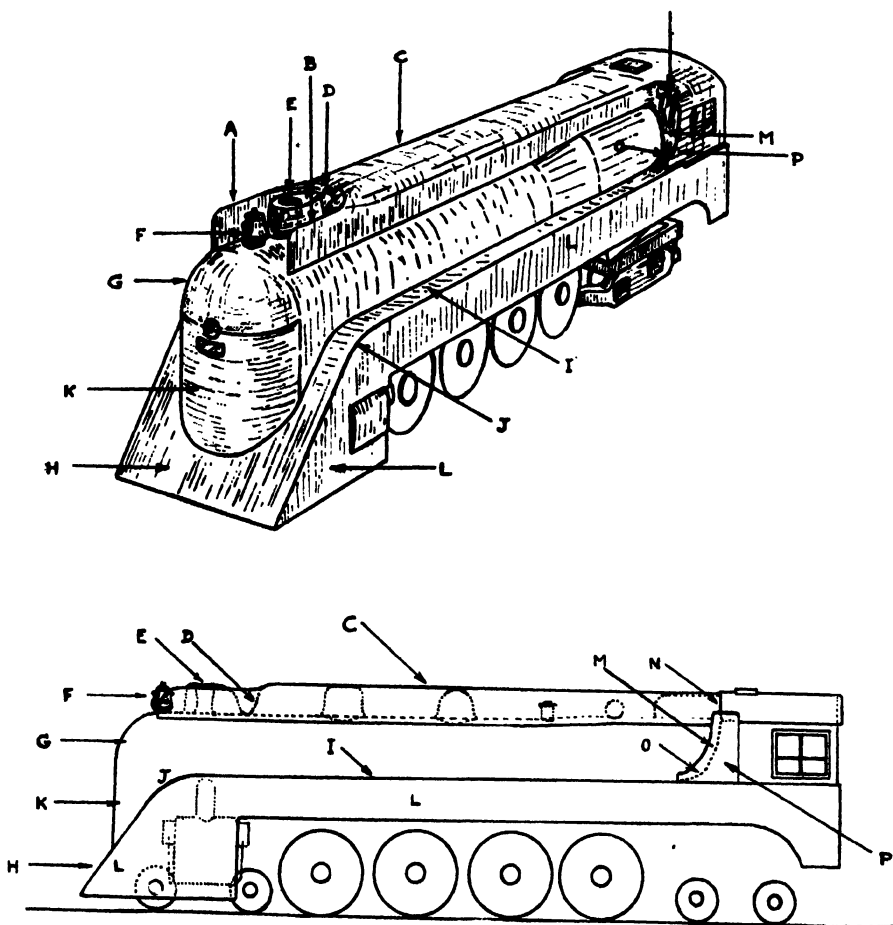


FIG. 21. Drawings of completely modified model. A, B, side plates on each side of stack. C, cowling over the dome, turrets, etc.; A, B, are continuations of the sides of C. D, smooth curved surface from boiler top to top of cowling C. E, streamlined smoke stack. F, bell edgewise to the wind in front of the stack. G, approximately quarter spherical nose on boiler. H, inclined plane replacing pilot on existing types (coupler not shown in drawing). I, running boards. J, rounded front of running boards. K, Smooth cylindrical front between G and H. L, side curtains over cylinders, valve motion, etc. M, new front to cab — sloping backwards from the generous curve between running board I and cab front M. N, Smooth rounded corner between cab roof and new cab front M. O, smooth protruding somewhat forward of the cab front M. P, side plates fitted to cab sides and

Conclusion

The model thus finally modified is shown in Figs. 18 and 19 and in the drawings of Fig. 21. It operates by inducing a layer of clean air under the smoke layer and by suitable cowling subsequently minimizes the intermingling of the smoke layer with the clean air layer. It introduces clean air along the boiler sides, which is ultimately swept upward in front of the cab windows.

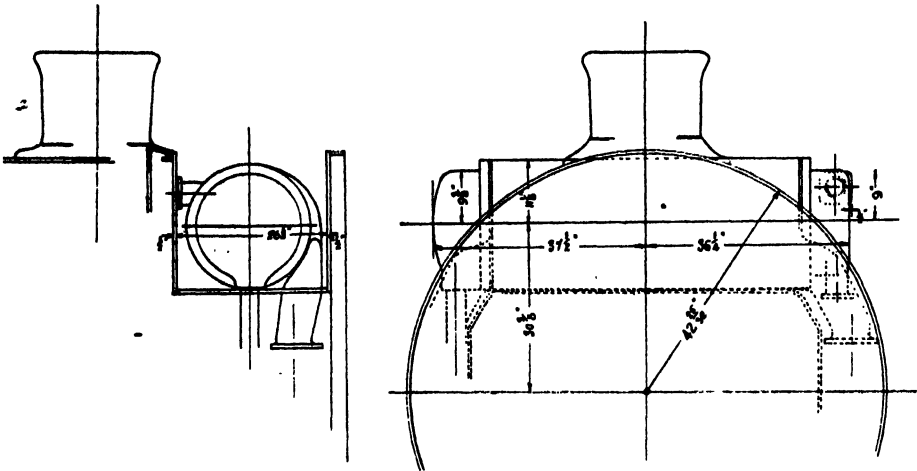


FIG. 22. *Location of feed-water heater.*

The modifications have been made with the idea of reducing turbulence and eddying and thereby cutting down the wind resistance as shown by the following results.

The value of $\frac{R}{V^2} \times 100$ has been reduced from 0.1089 to 0.0706, a reduction of some 35%.

The feed-water heater has been placed in a new position as a result of considerations made by the Operation Department of the Canadian National Railways. This position is just ahead of the stack and partly lowered into the smoke box as shown by Fig. 22, and Figs. 18 and 19.

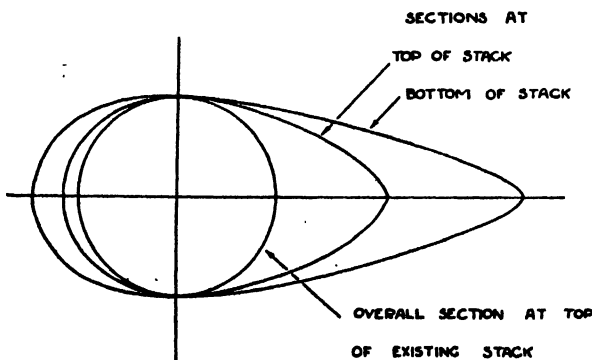


FIG. 23. *Sections of streamlined smoke stack.*

From the top surface of this feed-water heater, a level platform is taken back on either side of the stack and runs in a smooth curve into the cowling, over the dome and turrets.

Fig. 20 is a photograph of the silk streamers on the new model suspended in the tunnel with an air speed of 45 m.p.h. It forms an interesting contrast to the flow picture, Fig. 10, taken at the same speed with the original model.

The lifting of the smoke at the stack is clearly indicated by the streamers and the good flow over the top and sides of the boiler can be seen. The upward current of clean air over the cab window is also indicated.

Fig. 21 shows the developed model and indicates by letters the additions that must be made to existing types of locomotives to convert them to the improved type.

Application has been made for patents on the new design.

Acknowledgments

The author is grateful to Prof. J. H. Parkin, Assistant Director, Division of Physics, in charge of Aeronautics, for his assistance and valuable advice throughout the work and also to Mr. Brooks and Mr. Connal of the Operation Department of the Canadian National Railways, for their willing co-operation and the provision of photographs of a 6100 locomotive drifting, and a drawing showing the location of the feed-water heater which appear in this report.

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THE BREMIDAE OF MANITOBA¹

BY FERRIS NEAVE²

Abstract

The distributional ranges of many species of Bremidae meet or overlap in Manitoba. The distribution of most of these species is shown to follow closely the summer isotherms, which, in general, run in a northwest to southeast direction. Three main areas are recognized, each characterized by the presence or absence of certain species: (1) southern, (2) central, (3) northern and eastern. In the latter, three sub-areas are indicated. In all, 23 species of Bremidae are recorded from the province.

When Franklin (1) published his indispensable work on the "Bombidae of the New World", six species of bumble bees were known from Manitoba. He indicated his belief that five others would be found in this region. The list, thus amplified, included nine species of *Bremus* (*Bombus*) and two species of *Psithyrus*. He comments (1, p. 198) on the apparent scarcity of species in Manitoba and certain other regions, adding, however, that "this may be due largely to lack of extensive collecting in these portions of Canada."

At the present time the writer has records of 23 species (19 *Bremus*, 4 *Psithyrus*) within this territory and other additions may be expected, particularly in the extreme north of the province. This is a larger number than is recorded by Franklin from any North American state or province lying wholly east of the Rocky Mountains, and though recent years have added species to many of these local lists the Manitoba total is very significant in view of the intensive collecting which has gone on in districts farther south and east. It is now evident that, far from showing a deficiency in these insects, Manitoba holds a key position in the distribution of many species, being the meeting ground on which various forms, whose centres of distribution lie farther north, south, east or west, find their natural limits. The province therefore has no characteristic "Manitoban" fauna of bumble bees, but is a forum for representatives from adjacent regions.

General Considerations

The geography of this part of Canada is well adapted to a distributional study of this kind. Owing to the comparative lack of vertical relief, the complicated local conditions which are introduced by peaks and ranges are eliminated and it is possible to observe the broad general effects of climatic influences. The Bremidae are favorable subjects inasmuch as they are large conspicuous insects, easy to find and to collect, and possess strong powers of flight which enable them to surmount small local barriers. As a result of this ability, combined with the physical nature of the country, there appear to be no "islands" in the distribution of the various species, though further collecting in the Riding Mountain district may well disprove this idea.

¹ Manuscript received December 5, 1932.

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The regional distribution of certain American bumble bees has been the subject of a paper by Lutz (4) who, however, approaches the subject from a phylogenetic viewpoint and indicates the range of the various species only in a very broad way. Published records were collected together by Lutz and Cockerell (5) in 1920, and many locality records have been published more recently by Frison in his numerous papers on the family. None of these articles increase our knowledge of the Manitoba forms except one of Frison's (3) which contains records of six species collected at Cormorant Lake, near The Pas, by J. Russell.

The present writer has had opportunities during the last seven years to collect bumble bees in many parts of the province, and, in view of the dearth of published information, he has relied very largely upon these collections in drawing up the present account. The localities at which collections have been made (by the writer or others) are indicated on the accompanying map (Fig. 1). At few of these points can it be claimed that exhaustive search has been made. In some cases only the casual picking up of a few specimens was possible. Nevertheless, even these incomplete records are of great assistance in forming a general picture of the distribution of the species. A glance at the map will show that the chief gaps are in the far north and northeast and to a less extent along the southern border.

Areas Recognized

On the basis of the data presented on later pages it is necessary to divide the province into at least three main areas, as indicated on the map. These areas may be defined briefly as follows:

I. A relatively small southern area characterized by the presence of *B. fervidus*, *B. nevadensis* and *B. separatus*.

II. A large central area comprising most of the settled parts of Manitoba. No species (except *B. huntii* which cannot be considered at all characteristic) is strictly confined to this area which, however, is sharply delimited by the absence of certain species belonging to the other two areas.

III. A northern and eastern area, which, as at present constituted, covers by far the greater part of the territory under discussion. Species characteristic of this area are: *B. couperi*, *B. melanopygus*, *B. mixtus*, *B. flavifrons*, *B. pleuralis*, *B. frigidus*, *B. sylvicola*, *B. kirbyellus* and *P. fernaldae*. The area, however, even at the present time can be divided into certain sub-areas. The writer is of the opinion that it will be necessary in the future to recognize a fourth main region in the north and northeast. *B. kirbyellus* may belong to this group, which will probably include also certain far-northern species not yet recorded from the province, such as *B. arcticus*, *B. polaris* and *B. strenuus*. At present we have no data to show where the boundary of this last area should be drawn.

The sub-areas can be diagnosed briefly:—

(A) A strip of country not more than 50 miles wide immediately adjacent to Area II and extending completely across the province. This zone is

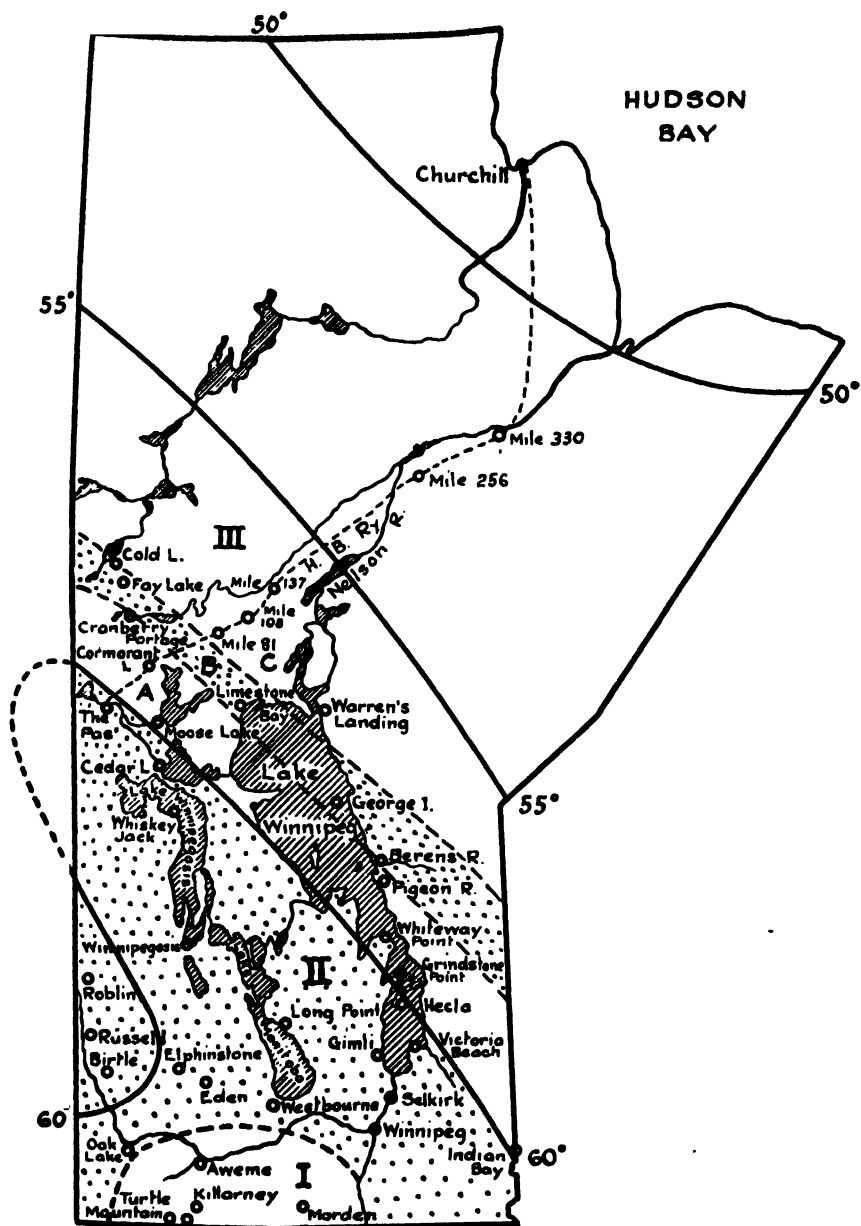


FIG. 1. Sketch map of Manitoba showing localities at which collections have been made, summer isotherms and areas recognized in distribution of *Bremidae*.

inhabited by *B. couperi* and *B. melanopygus*, but apparently not by the other species mentioned above.

(B) A similar narrow strip in which *B. mixtus* and *P. fernaldae* are present.

(C) Characterized by the presence of *B. pleuralis* and *B. frigidus*. The northeast boundary of this sub-area cannot be defined at the present time.

The numbers of species recorded at present from each main area are:—Area I, 9; Area II, 9; Area III, 17.

It is strikingly evident that the northeast part of the province is by far the richest in number of species, despite the fact that less collecting has been done in this area than in any other. It is obvious from these figures that many species are found in at least two of the areas. Nevertheless, only one species (*B. terricola*) occurs throughout the whole of the territory from which records are available. *P. ashtoni* is probably inquiline upon *B. terricola* and may be nearly co-extensive. *B. borealis*, *B. rufocinctus*, *B. ternarius* and *B. vagans* cover the whole of Areas I and II and range at least a little way into III, but are certainly not characteristic of the latter. *P. laboriosus*, which has been recorded as an inquiline upon *B. vagans*, probably has an equally wide distribution, though not yet reported from Area I. Some of the northern species also range over a vast territory, though this is not considered herein to represent more than one area. On the other hand, the species listed as characteristic of Area I have a very circumscribed distribution in the province, though a wide range outside it. *B. couperi*, again, is present (and quite common) in a narrow zone and, judging from published records, this may be true of its distribution in other parts of the country. A very similar altitudinal zoning of some of the same species is found in the Canadian Rockies (6). *B. borealis* and *B. ternarius* occur in the eastern foothills, while *B. kirbyellus*, *B. melanopygus*, *B. mixtus*, *B. sylvicola* and *P. fernaldae* occupy a high zone. There are, however, some minor differences. *B. terricola*, for example, appears to be somewhat more restricted in its vertical range than would be indicated by its horizontal distribution in Manitoba.

Factors Influencing Distribution

Doubtless there are various factors which play a part in controlling the distribution of these insects. Humidity, competition between species, and the character of the flora suggest themselves as probable influences. Temperature however appears to be the most obvious factor concerned. The accompanying map (Fig. 1) shows a striking correspondence between the geographical distribution of the species and the direction taken by the summer isotherms.* Further evidence of the importance of the temperature factor is obtained by considering the distribution of the various species outside the province. Of all the species listed here, it is probable that only one (*B. terricola*) can be considered to have its centre of distribution in or near Manitoba. The rest, or most of them, judging by published records, have their distributional centres at a considerable distance from this area. An examination of the available data shows that most of these centres lie either to the north and northwest or to the south and southeast. About 15 species can be located in these two directions. Only two species (*B. huntii* and *B. nevadensis*) can be classed as western and southwestern forms and it is doubtful whether any species represents invasion from the northeast. The explanation seems to be

* Isotherms according to Atlas, Department of the Interior, Canada, 1906.

that the distribution follows closely along the isotherms, which run in a north-west to southeast direction. Extension of range from southwest to northeast, or *vice versa*, would involve cutting across the isotherms.

Although the importance of temperature is thus readily to be seen, the question remains as to whether its effect is mainly a direct one, *i.e.*, exerted on the bees themselves, or an indirect influence working through modifications of the vegetation. The writer believes that the direct effect is considerable. This is indicated by differences in the length and texture of the hair between northern and southern forms. Further, the distributional limits of many species do not seem to coincide with any major changes in the type of vegetation,—in other words, these bees appear to be more sensitive than many of the plants. On the other hand, the boundary between Areas I and II does correspond very closely with the edge of the coniferous forests. Also, it may be noted that the slightly colder district in the western part of Area II shows no special Bremid features except perhaps in the presence of *B. huntii*. The vegetation in this district is similar, in its larger features, to adjoining parts of Area II. The flora, no doubt, is a strong factor in distribution and further work may show its importance in cases where its influence cannot be demonstrated at the present time.

Notes On The Species

In the following list, an asterisk denotes a new record for the province of Manitoba. Records which are not credited to any collector are due to the writer.

**Bremus separatus* Cress.

Confined to the southern part of the province. The only localities so far are Aweme (N. Criddle, also R. D. Bird) and Turtle Mountain. It may probably be found to occupy most of the area south of the Assiniboine valley and west of the Red River.

Bremus rufocinctus Cress.

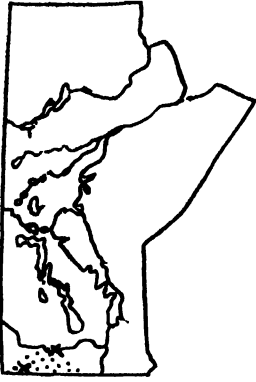
Common throughout I and II and extending a little way into III. The most northerly records are Berens River in the east and Moose Lake in the west. The color variety *albertensis* Ckll. has an equally wide range.

**Bremus nevadensis* Cress.

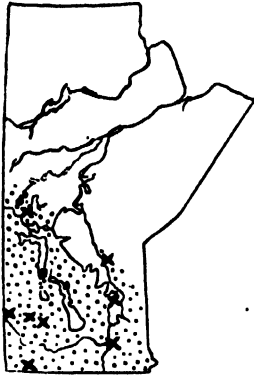
This handsome species has been found only in the south of the province,—Aweme (R. D. Bird) and Turtle Mountain. Its occurrence here extends its known range in a northeasterly direction.

**Bremus fervidus* F.

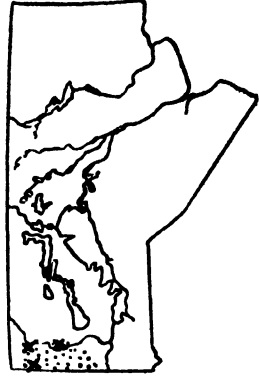
Franklin (1, p. 392) says "almost certainly present in Manitoba", but its range within the province appears to be limited to about the same area as *B. separatus*; Aweme (N. Criddle, also R. D. Bird); Oak Lake (B. Sharman); Turtle Mountain.



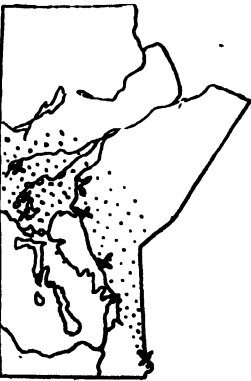
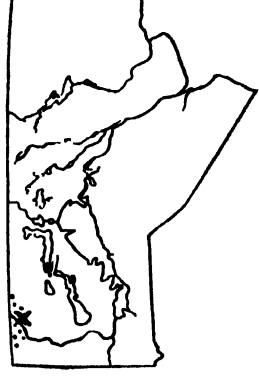
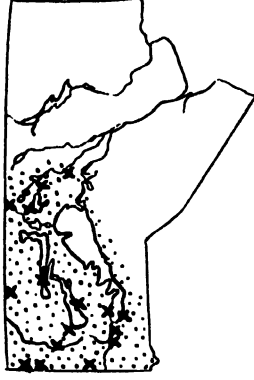
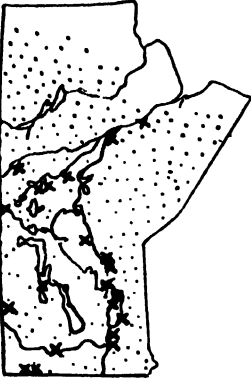
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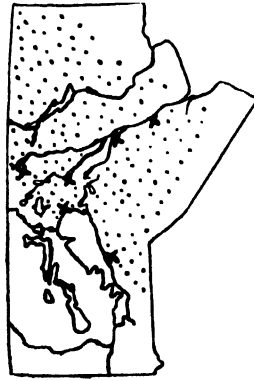
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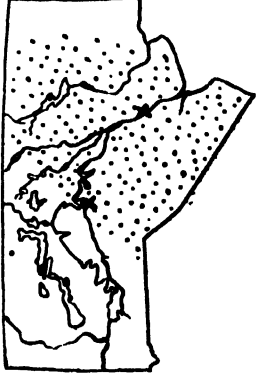
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FIG. 2. The crosses indicate actual locality records. The stippling indicates areas in which the species is expected to occur. In filling in the latter the known distribution of the species outside the province is taken into consideration. 1, *B. nevadensis*, *B. separatus*. 2, *B. rufocinctus*. 3, *B. fervidus*. 4, *B. terricola*. 5, *B. ternarius*. 6, *B. huntii*. 7, *B. melanopygus*. 8, *B. mixtus*. 9, *B. frigidus*.

Bremus terricola K.

As already indicated, this is the most generally abundant species. It appears to be equally common from the southern boundary to the most northerly locality at which collections have been made (Mile 330, Hudson Bay Railway) and it probably extends considerably farther north, as it is recorded from "Hudson Bay Territory" and "Lat. 65° North America."

Bremus bifarius Cress.

Reported only from Cormorant Lake, near The Pas (J. Russell; Frison (3, p. 105).

Bremus ternarius Say

This is probably the commonest species throughout most of the settled parts of Manitoba. It is less plentiful in the north and east but extends as far as Mile 137, H. B. Ry. The queens do not appear so early in spring as those of *B. terricola*.

**Bremus huntii* Greene

This species reaches Manitoba at the extreme eastern edge of its range. The only record to date is a single queen taken at Birtle, 31.V.23 (R. D. Bird). It should be found at other points in the west of the province, but is probably not common.

**Bremus sylvicola* K.

Probably widespread in the far north of the province. The writer's only record is from Cold Lake, 90 miles north of The Pas, 9.VIII.31. This is the most southerly record for the species in this part of its range.

Bremus melanopygus Nyl.

The local distribution of this species is of great interest. Lutz (4; p. 515) says: "ranging from southern Alaska to Baffin Land, south to Colorado but not east of Saskatchewan in the southern part of its territory." It is true that it does not occur in southwestern Manitoba. Frison records it from Cormorant Lake, near The Pas (J. Russell) and it is undoubtedly common throughout northern Manitoba, as the writer has specimens from Cold Lake and also Moose Lake and Warren's Landing at the north end of Lake Winnipeg. From this territory it extends in a southeasterly direction to the southeast corner of the province, where the writer has taken it at Indian Bay, on the Manitoba-Ontario boundary. The writer also found it at Kenora, Ontario. These localities extend the known range of the species some 400 miles in a southeasterly direction. The southwest boundary of its range in Manitoba coincides very closely with the summer isotherm of 60° as represented on the map. This line also represents approximately the southwest limits of *B. couperi*, with which *B. melanopygus* is frequently associated. *B. couperi*, however, does not extend so far north as the present species. It will be interesting to find the distributional limits of *B. melanopygus* in Ontario and in northern Minnesota, where it should almost certainly occur.

It is frequently found on *Salix* in spring and appears to be an early species, at least in the southern part of its range. At Gull Harbor, Lake Winnipeg, the writer has taken workers as early as May 27.

**Bremus mixtus* Cress.

Another boreal form, which does not extend quite so far south as *melanopygus* but is characteristic of Area III-B and farther north. Southernmost records are Berens River and Limestone Bay. Further north, the writer has taken it at Fay Lake and at points on the H. B. Ry. between Mile 137 and Mile 327.

**Bremus frigidus* F. Smith

This form, which ranges from Alaska to Labrador, extends as far south as Warren's Landing at the north end of Lake Winnipeg. It has also been taken at Mile 330, H. B. Ry., indicating a wide distribution in the north of the province.

Bremus couperi Cress.

Frison (3, p. 114 *et seq.*) considers this form to be a variety of *frigidus*, with which it is "structurally identical." Without necessarily implying disagreement with this view, the present writer is keeping the two forms apart as a matter of convenience. In a distributional study they have to be treated separately, as their geographical ranges do not coincide, though they overlap to a small extent. In Manitoba, at any rate, the two forms appear to be always readily distinguishable.

As already indicated, *couperi* is absent from a large area in the south and west. It is particularly common along most of the shoreline of Lake Winnipeg and is plentiful even on small islands such as George Island, which is situated ten miles from the nearest mainland. It does not appear to range into the far north, though it occurs at Mile 108, H. B. Ry. The localities to date indicate a rather narrow range within the province. Both light-tailed and black-tailed forms are common in this zone.

Bremus perplexus Cress.

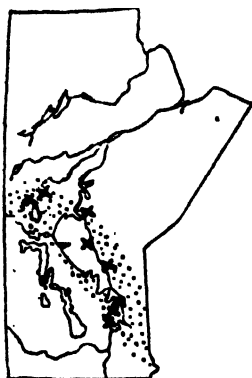
The range of this species is somewhat peculiar and does not seem to be determined by the same factors as most of the other Bremidae of the province. It is found, not very abundantly, over a fairly large area in eastern Manitoba. Winnipeg (J. B. Wallis, also F. N.); Gull Harbor; Victoria Beach; Berens River; Pigeon River. The species probably finds its northwestern limit in Manitoba.

Bremus vagans F. Smith

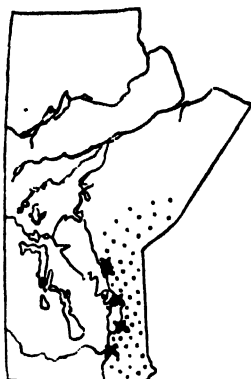
The distribution of this species corresponds very closely with that of *B. ternarius*. It is common over Areas I, II and III-A. The most northerly records are Pigeon River in the east and Cranberry Portage in the west.

**Bremus flavifrons* Cress.

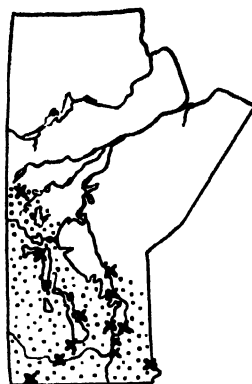
This northern form is known so far only from Cold Lake, 9.VIII.31. It has been recorded from both east and west shores of Hudson Bay and will probably be found widely distributed in northern Manitoba.



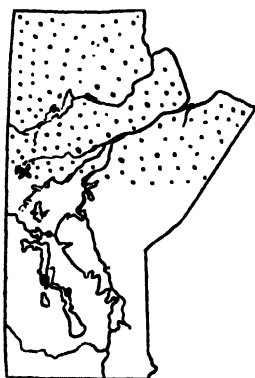
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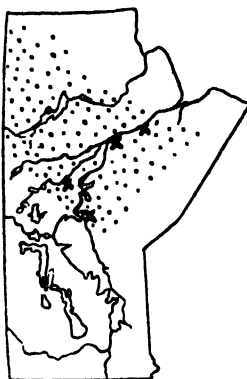
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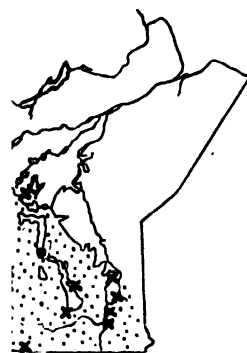
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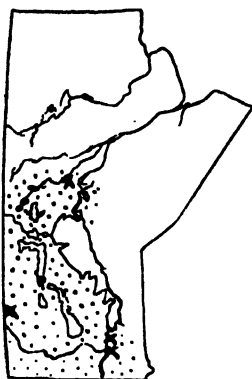
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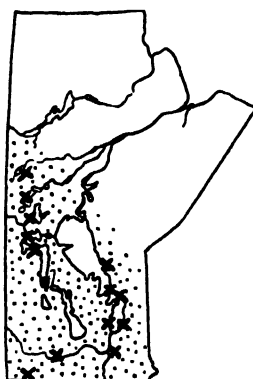
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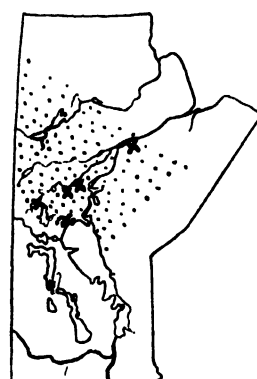
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FIG. 3. The crosses indicate actual locality records. The stippling indicates areas in which the species is expected to occur. In filling in the latter the known distribution of the species outside the province is taken into consideration. 10, *B. couperi*. 11, *B. perplexus*. 12, *B. vagans*. 13, *B. flavifrons*, *B. sylvicola*. 14, *B. pleuralis*. 15, *B. borealis*. 16, *P. laboriosus*. 17, *P. ashtoni*. 18, *P. fernaldae*.

**Bremus pleuralis* Nyl.

Franklin records this species from Alaska only. More recently it has been reported from Yukon Territory, the mountains of Arizona, British Columbia and Fort Norman, west of Great Bear Lake. Its occurrence in northern Manitoba is therefore a very great extension of its known range*. Specimens collected by the writer, however, conform entirely to the specifications laid down for *pleuralis*, namely, a pure yellow band on the thorax and a sharply defined black inter-alar band. In addition, certain workers show the characteristic dark face. All are of the red variety which resembles *flavifrons* and *centralis*. The localities are: Warren's Landing; Mile 137, Mile 256 and Mile 330, H. B. Ry. These points indicate a wide zone of distribution.

**Bremus kirbyellus* Curt.

Nelson River, at junction of Butnau River, 1.IX.28, one male.

Bremus borealis K.

Though not very abundant, this species occurs throughout the whole of Areas I and II. The northernmost record is Moose Lake.

**Psithyrus laboriosus* F.

Not common but widely distributed; Winnipeg (J. B. Wallis, also F. N.); Selkirk; Russell; Mile 137, H. B. Ry.

Psithyrus insularis F. Smith

Cormorant Lake, 1.VII.27 (J. Russell), Frison (3).

Psithyrus ashtoni Cress.

Common throughout all the southern and central parts of the province. The writer's northernmost record is Cold Lake, but if, as is probable, this species is inquiline on *B. terricola* it may be expected much farther north than this.

Psithyrus fernaldae Franklin

Found so far only in northern Manitoba, though further collecting will probably extend its range in the east of the province. Frison records it from Cormorant Lake (J. Russell) and the writer has taken it at Limestone Bay and along the H. B. Ry., Mile 108 to Mile 260. Both in Manitoba and at Jasper, Alta., it appears to be associated with members of the subgenus *Pratobombus*.

Frison (2) recognizes nine subgenera in the North American species of *Bremus*, namely: *Fraternobombus* Skorikov, *Nevadensibombus* Skorikov, *Separatobombus* Frison, *Cullumanobombus* Vogt, *Alpinobombus* Kruger, *Terrestribombus* Vogt, *Pratobombus* Vogt, *Subterraneobombus* Vogt, *Fervidobombus* Skorikov. Also three subgenera of *Psithyrus*: *Laboriopsithyrus* Frison,

* It is interesting to note that Fort Norman, though 1200 miles away, has the same summer temperature as the more southerly Manitoban localities for the species.

Ashtonipsithyrus Frison and *Fernaldaepsithyrus* Frison. While these groupings are not indicated in the foregoing treatment of the species, it may be mentioned that each of these subgenera except *Fraternobombus* is present in Manitoba. Very few of the political divisions of North America have a more representative fauna of bumble bees.

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DECOMPOSITION AND MOVEMENT OF HERBICIDES IN SOILS, AND EFFECTS ON SOIL MICROBIOLOGICAL ACTIVITY AND SUBSEQUENT CROP GROWTH¹

BY W. E. BOWSER² AND J. D. NEWTON³

Abstract

Experiments were conducted to determine the residual effect of sulphuric acid, copper sulphate, sodium chlorate, barium chlorate and sodium dichromate on three typical Alberta soils. No problem of residual effect was found with the two leaf sprays, sulphuric acid and copper sulphate. Sodium chlorate will remain toxic over a period of about two years, depending mainly on the organic matter content of the soil and the amount of leaching that takes place. The distance that the chlorate will leach down into the subsoil will depend on the amount of rainfall and the character of the soil. As soon as the chlorate is leached out or is reduced the soil returns to its normal productive power. Sodium dichromate decomposes very rapidly in the soil. It has a depressing effect on nitrification and on the activity of the soil micro-organisms. However, there is practically no residual effect from the use of sodium dichromate on soils of medium to high organic matter content.

Introduction

The use of chemicals for the destruction of weeds has made it necessary to determine the effects of these herbicides on subsequent crop growth. The effects will depend, in part at least, upon such factors as rate of movement of the herbicide in soils, rate of decomposition, and effect on soil microbiological activity. Thus, important soil problems have been created, and the purpose of this work was, therefore, to investigate the nature of the effect of weed herbicides on the soil.

Sulphuric acid, copper sulphate, sodium chlorate and sodium dichromate were investigated in relation to their effect on the soil processes and subsequent crops. A preliminary experiment with barium chlorate is also included. The chlorates and chromates act, at least in part, through the soil and results to date have definitely proved that a residual effect on the soil creates a problem.

In the experiments conducted three concentrations of pure chemical were used. These fairly well covered the range used for practical purposes. The concentrations are designated in this paper as Check, Concentration A, Concentration B and Concentration C. Table I shows the concentrations of the various chemicals used.

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TABLE I

RATES OF APPLICATIONS OF CHEMICALS TO SOIL REPRESENTED BY TREATMENTS
A, B, C, ETC.

Check		A -		A		B		C		C +	
		Gm. per 100 gm.	Lb. per acre	Gm. per 100 gm.	Lb. per acre	Gm. per 100 gm.	Lb. per acre	Gm. per 100 gm.	Lb. per acre	Gm. per 100 gm.	Lb. per acre
H ₂ SO ₄	None	—	—	.0024	30	.004	50	.008	100	—	—
CuSO ₄	None	—	—	.0012	15	.0024	30	.0048	60	—	—
NaClO ₃	None	—	—	.026	327	.052	654	.104	1308	—	—
Na ₂ Cr ₂ O ₇	None	.013	163	.026	327	.052	654	.104	1308	.146	1838
Ba(ClO ₃) ₂	None	—	—	.039	489	.078	978	.156	1960	—	—

In most of the work three types of soil were used: Edmonton black, a rich black loam high in organic matter; Breton grey, a typical leached wooded soil; and Winterburn fine sandy loam, a soil of intermediate fertility. Duplicate crocks of these soils were used in all greenhouse experiments. The field experiments were conducted on plots in Belgravia field by permission of the Field Crops Department, University of Alberta. The plots were of even topography and of typical Edmonton black soil. The field had been fallowed the year previous to the beginning of the work. All field plots were in quadruplicate (see Fig. 1).

With the exception of two experiments all pot work was kept in a greenhouse at optimum moisture and cultivated at regular intervals. Two sets of experiments, one with sodium chlorate and one with sodium dichromate, to note their effect on microbiological activity, were kept at optimum moisture and maintained at one temperature in a laboratory control chamber.

In testing the residual effect on crop growth, wheat was the only plant used.

Methods

Chlorates

Chlorates were determined by the following method: the soil filtrate was boiled for 15 min. with standard ferrous sulphate and the excess titrated with standard potassium permanganate. This gave quite accurate results. The determination had to be carried out fairly quickly as the organic matter in the filtrate slowly reduced the potassium permanganate, and consequently reduced the chlorate readings. The first end-point reached was the correct one. This method is a modification of that of Treadwell and Hall (17, p. 398). It was the only one found satisfactory for such small amounts and applicable to the soil solution.

By adding flocculents it was found that a higher chlorate reading was obtained than by using a soil filtrate to which no precipitating agents had been added. This higher reading is undoubtedly due to less organic matter present in the filtrate.

It was found that the soils varied in the amount of chlorate recoverable. Using no precipitants, the following amounts of chlorate (expressed as percentage of chlorate added) could immediately be extracted by shaking for

	Fallow	Check	Wheat	Check	Plot 1/4 acre 0 x 50 nks ↑ Path 3 links
		CuSO ₄ Conc. C		NaClO ₃ Conc. C	
		CuSO ₄ Conc. B		NaClO ₃ Conc. B	
		CuSO ₄ Conc. A		NaClO ₃ Conc. A	
		Check		Check	
		CuSO ₄ Conc. C		NaClO ₃ Conc. C	
		CuSO ₄ Conc. B		NaClO ₃ Conc. B	
		CuSO ₄ Conc. A		NaClO ₃ Conc. A	
		Check		Check	
		CuSO ₄ Conc. C		NaClO ₃ Conc. C	
	Fallow	CuSO ₄ Conc. B	Wheat	NaClO ₃ Conc. B	
		CuSO ₄ Conc. A		NaClO ₃ Conc. A	
		Check		Check	
		CuSO ₄ Conc. C		NaClO ₃ Conc. C	
		CuSO ₄ Conc. B		NaClO ₃ Conc. B	
		CuSO ₄ Conc. A		NaClO ₃ Conc. A	
		Check		Check	
		CuSO ₄ Conc. C		NaClO ₃ Conc. C	
		CuSO ₄ Conc. B		NaClO ₃ Conc. B	
		CuSO ₄ Conc. A		NaClO ₃ Conc. A	
Check		Check		Check	
CuSO ₄ Conc. C		NaClO ₃ Conc. C		H ₂ SO ₄ Conc. C	
CuSO ₄ Conc. B		NaClO ₃ Conc. B		H ₂ SO ₄ Conc. B	
CuSO ₄ Conc. A		NaClO ₃ Conc. A		H ₂ SO ₄ Conc. A	
Check		Check		Check	
CuSO ₄ Conc. C		NaClO ₃ Conc. C		H ₂ SO ₄ Conc. C	
CuSO ₄ Conc. B		NaClO ₃ Conc. B		H ₂ SO ₄ Conc. B	
CuSO ₄ Conc. A		NaClO ₃ Conc. A		H ₂ SO ₄ Conc. A	
Check		Check		Check	
CuSO ₄ Conc. C		NaClO ₃ Conc. C		H ₂ SO ₄ Conc. C	
CuSO ₄ Conc. B		NaClO ₃ Conc. B		H ₂ SO ₄ Conc. B	
CuSO ₄ Conc. A		NaClO ₃ Conc. A		H ₂ SO ₄ Conc. A	
Check		Check		Check	
CuSO ₄ Conc. C		NaClO ₃ Conc. C		H ₂ SO ₄ Conc. C	
CuSO ₄ Conc. B		NaClO ₃ Conc. B		H ₂ SO ₄ Conc. B	
CuSO ₄ Conc. A		NaClO ₃ Conc. A		H ₂ SO ₄ Conc. A	
Check		Check		Check	

FIG. 1. Plan of Edmonton field plot experiments with sulphuric acid, sodium chlorate and copper sulphate in 1931.

one hour: from fine sandy loam, 80; from Edmonton black, 71; and from wooded grey soil, 90%. By adding copper sulphate, carbon black, magnesium carbonate and calcium hydroxide these values were increased to 90, 88 and 94%, respectively.

Dilute phosphoric acid was added to prevent action of the chloride ion on the potassium permanganate. Silver nitrate was added to the reduced and unreduced solutions, and the increased opalescence of the reduced samples showed that what was being measured was chlorates and not oxidation products of their decomposition.

The only other method found at all feasible was the silver nitrate method after reduction of the chlorate with ferrous sulphate, but even this was unsatisfactory. The amount of chloride was too small to weigh accurately and difficulty in the chromate titration method was encountered, due to the reaction of the reagents used in the reduction.

Nitrates

It was found impossible to determine nitrates in the presence of chlorate by Harper's (7) standard phenoldisulphonic acid method. The chlorate has an effect on the easily oxidized reagent, producing color tints that make an accurate reading impossible.

In most determinations the nitrates were in such minute quantities that titration of nitrates reduced to ammonia was impractical. The only satisfactory method that could be found for measuring the ammonia was Nesslerization. The difficulty was to measure accurately the small differences in soil nitrates due to the chlorate added. Scales' (16) method seemed to be the most satisfactory one to use. The procedure as developed is given below. A clear soil filtrate, one-tenth normal with sodium hydroxide was reduced with a zinc-copper couple. The distillate was treated with Nessler reagent and measured in Nessler tubes. It was found most satisfactory to obtain three different distillates; one where the alkaline solution was immediately reduced and distilled with the metal couple, one where the alkaline solution was boiled and distilled without the metal, and one where the boiled solution was then reduced and distilled with the metal couple. Since it was supposed that the differences found would be small, the object was to eliminate as far as possible the error due to free soil ammonia, ammonia from soil organic matter and the reduction of soil nitrates. Weighing the Nessler precipitate could not be expected to give accurate differences, but could sometimes be used as a check on the color readings. Considerable time was spent in standardizing and applying the above method.

For the determination of nitrates in the presence of chromates the phenoldisulphonic acid method was used. The chromate in the solution was reduced by ferrous sulphate, in the cold. The solution was then made very slightly alkaline and the precipitated iron salts filtered off. The solution could then be evaporated and the determination carried on by Harper's method. Care had to be exercised to prevent loss of nitrates by the vigorous reaction between the phenoldisulphonic acid and the excess of salts present.

Chromates

Chromates were determined by the potassium ferricyanide spot plate method as outlined by Treadwell and Hall (17, p. 548). The soil filtrate was titrated with standard ferrous sulphate using potassium ferricyanide as an outside indicator.

Micro-organisms

The numbers of bacteria and fungi were determined by the plate count method. A nutrient agar medium recommended by Fred and Waksman (5, p. 9, Medium 4) was used for the bacterial plates, and an acid agar medium recommended by Waksman (5, p. 13, Medium 18) for the fungi plates.

Experimental Results

Sulphuric Acid

Sulphuric acid is used as a leaf spray, generally for the control of annual weeds. Its lethal action is on the exposed part of the plant. Åslander (3) suggests that sulphuric acid does not move in the plant and its effect is dependent on the amount of surface the spray can cover. The applications recommended are from 30 to 100 lb. per acre. It is seen then that the amount of acid that reaches the soil is necessarily very small. One of the authors (15) found in Edmonton black soil that one ton of sulphur reduced the pH from 6.5 to 5.2 and in Breton grey podsolized soil from 6.7 to 5.1. This would suggest that the amount applied in the sulphuric acid experiments would have little or no residual effect on the soil.

A series of eight one-gallon crocks of each of the three following soils was placed in the greenhouse: Winterburn fine sandy loam, Edmonton black and Breton grey loam. Three different rates of application of sulphuric acid were used (see Table I), and all treatments and checks were in duplicate. A series of plots was laid out in the field in quadruplicate (see Fig. 1). These plots were treated with sulphuric acid on July 2, 1930. They remained fallow that year and were sown with wheat in 1931.

Nitrates and micro-organisms were determined periodically in the plots and crocks in 1930. The results were rather irregular but there appeared to be a very slight depression of the nitrates and fungi numbers late in the season in the field plots treated with sulphuric acid. By September 5 the nitrates on the check plots had increased from 30 to 36 p.p.m.,* while on the plots receiving Concentration C they had decreased from 28 to 21 p.p.m. However, it appears that there was no important detrimental effect on the soil biological activity as a result of the sulphuric acid application.

A crop of wheat sown in the crocks after six months showed no ill effect from the acid treatment. A slight stimulating effect due to the acid was noticeable in the poorer soils; *i.e.*, in the Winterburn fine sandy loam and the Breton grey.

The plots were sown with wheat the following spring, in May 1931. No apparent differences were seen in the growing crop. Threshed grain yields on the plots are given in Table II. No definite trend is noted and the differences in yield are not really significant.

p.p.m. = parts per million.

Copper Sulphate

As a result of the comparative success with copper sulphate as a chemical for the destruction of annual weeds it was decided in 1931 to test its residual effect. With copper sulphate, as with sulphuric acid, it was felt that the amount added was too small to have any detrimental residual effect on the soil. Bourcart (4) says, "The soil is capable of absorbing enormous quantities of copper sulphate without the latter injuring the plant". He also suggests that this is due to the formation of the more insoluble and less toxic copper salts by reaction with the soil. This is emphasized by Marshall (12, p. 292) who points out that with metallic salts the toxic action will depend mainly on the metal in solution. A series of 24 crocks similar to that used for the sulphuric acid treatment was placed in the greenhouse. The treatments were as stated in Table I. The crocks were maintained in the greenhouse at optimum moisture and cultivated every two weeks.

Nitrates were determined monthly for four months by the phenoldisulphonic acid method (see Table III). Although no definite trend can be noted it appears as if there was some stimulation in the richer soils due to the copper sulphate. This stimulation lasted only a short time after application. It also appears as if the weakest concentration stimulated sooner than the next higher concentration. Greaves' (6) work on the effect of salts on soil micro-organisms shows that a stimulation can be expected in weaker concentrations of a toxic salt. This is what appears to have happened with the addition of copper sulphate in small amounts.

TABLE II
WHEAT YIELDS OF EDMONTON FIELD PLOTS 1931; STRAW IN TONS PER ACRE,
WHEAT IN BU. PER ACRE

Treatment		Date of application		
		July, 1930	June, 1931	July, 1930
		H ₂ SO ₄	CuSO ₄	NaClO ₃
Ch.	Straw	3.95	4.06	3.88
	Grain	55.3	51.9	50.1
	% Grain	33.8	31.2	31.8
A	Straw	3.63	4.29	.88
	Grain	50.5	57.3	7.3
	% Grain	33.7	33.1	19.3
B	Straw	3.37	4.04	.51
	Grain	45.3	56.9	1.7
	% Grain	32.8	34.3	7.8
C	Straw	3.55	3.70	.17
	Grain	50.5	52.3	.02
	% Grain	34.6	34.7	1.2

NOTE: The sulphuric acid applied in 1930 and the copper sulphate applied in 1931 had little effect on the 1931 wheat yields, but the sodium chlorate applied in 1930 had a very great effect on 1931 wheat yields.

See Table I for rates of application represented by Treatments A, B, and C. Each yield figure represents an average of four plots.

TABLE III

NITRATES (P.P.M.) IN POT CULTURES TREATED WITH COPPER SULPHATE, 1931

	March 3	May 9	June 6	June 30	Aug. 14
Wooded Ch.	Trace	36	31	27	40
“ A	“	32	46	29	36
“ B	“	33	35.5	27	24
“ C	“	35	26	37	23.5
Fine Sandy Loam Ch.	7.2	44	64	57	36
“ “ A	7.2	48.5	70	39	73
“ “ B	7.2	42	100	44	57
“ “ C	7.2	38	35	40	133
E. Black Ch.	24	50	70	99	182
A	24	61.5	80	100	160
B	24	70	94	88	159
C	24	52	67	100	133

NOTE: The nitrates show no definite trend following the application of copper sulphate.

Six months after treatment with the sulphate the crocks were sown with wheat. No difference was noted in germination or in subsequent growth.

On June 26, copper sulphate was applied to a series of plots of growing wheat. There was a heavy rain 12 hr. after application. Two days later considerable burning of the wheat leaves was observed. The plots receiving 60 lb. per acre appeared quite brown. As the wheat neared maturity the check plots lodged badly, while those receiving sulphate remained upright. The yields of wheat on these plots are given in Table II.

These figures show a small but definite increase in the ratio of grain to straw with the application of copper sulphate. A stimulation due to Concentration A and a depression due to Concentration C might also be noted. However, neither one of these is significant. If the check plots had not lodged they would, probably, have given a significantly higher yield than the treated plots.

A series of fallow plots was treated on June 26, 1931. The copper sulphate had a lethal effect on the growing weeds at the time of application. However, it did not have any apparent residual effect on any growth appearing after its application.

Sodium Chlorate

Chlorates are used mainly for the eradication of perennial weeds because their toxic action appears to persist over a much greater period of time than that of the leaf sprays, and chlorates therefore present the greater residual effect problem. Sodium chlorate was used as the type example and the major part of this work was devoted to its study.

The experiments conducted have shown that sodium chlorate affects certain normal soil processes to a limited extent. It has not been possible, however, from this investigation to explain why it acts in this manner.

Up to the present there are no definite data as to the cause of toxic action of chlorates*. Neller (14) says it is possible that the slower action and the higher toxicity of sodium chlorate are due to its gradual decomposition within the plant tissues, accompanied by the liberation of nascent oxygen. Neller also suggests that the chlorates lower the catalase activity of the roots. Loomis, Bissey and Smith (11) believe that the herbicidal action consists of

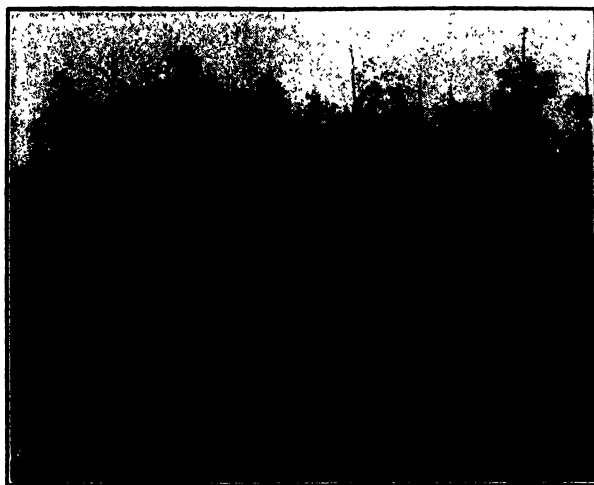


FIG. 2. *Wheat on field plots the year following treatment with sodium chlorate.*

both direct killing of underground portions of the plant and of a translocation to, and slow killing of, new sprouts. Latshaw and Zahnley (10) suggest that it interferes with photosynthesis and compels the plant to draw upon the root reserves until they are exhausted and die. However, Loomis, Bissey and Smith (11) say its action can go on in the absence of ultra-violet light. Although our results show that the

chlorate has a definite effect on microbiological processes in the soil it appears quite evident that its toxic action is not from that source. Loomis, Bissey and Smith (11) and Åslander (2) suggest that the chlorate is more effective if applied to the soil. It seemed reasonable, therefore, to suggest that a study of its effect on the soil and its movement in the soil is important.

A series of plots in quadruplicate was laid out in the spring of 1930. These were treated July 28, 1930, with sodium chlorate according to Table I. Bacteria and fungi determinations were made at intervals during the year. The numbers are rather irregular and show no definite trend following the application of chlorates. Nitrates were not determined that summer, as no satisfactory method of determining nitrates in the presence of chlorates was at that time standardized.

In the spring of 1931 the plots were sown with wheat. Chlorate damage was noticed with the first appearance of the shoots. The plots receiving the heaviest application were practically barren. What few seeds germinated or appeared above the surface survived only a short time. Any growth supported by these plots, Concentration C, germinated almost a month later. Fig. 2 shows the appearance of the plots shortly before harvest. The yields of wheat are shown in Table II and indicate the graded or corresponding effects of the different concentrations applied. All applications of chlorates materially

* The writers have been unable to find any published article that deals directly with the effect of the oxidising chemicals on the soil.

reduced the yield. The plot receiving Concentration A, 327 lb. per acre, gave a yield of 7 bu. per acre as against a 50-bu. yield on the check plot. Concentration B and Concentration C reduced the yield to practically nothing. Concentration B, 654 lb. per acre, approximates the average application recommended for the control of perennial weeds. It appears that under climatic conditions such as prevailed in 1930, Edmonton black soil, treated with 600 lb. of sodium chlorate per acre, will inhibit the growth of wheat for at least one year. The 1930 season was relatively dry with a long open dry fall. Although no analyses were made it may be supposed that in the spring of 1931 the chlorate was still concentrated quite close to the surface. It was, therefore, quite toxic to the young plants. These results are similar to the findings of Arny, Bridgford and Dunham (1). They state that when from 600 to 800 lb. per acre were applied the soil often remained sterile for a year. Megee and Lipscomb (13) state that the residual effect of a spring application usually disappeared by September. The length of time that chlorates will remain in the soil, toxic to the plant, seems to be a point upon which many different opinions are expressed. The investigations indicate that although the fertility of the soil and its percentage of organic matter are factors, rainfall and leaching are more important because they affect its distribution in the soil and therefore its toxicity to the plant.

On August 18, 1931, the upper three feet of soil were sampled and analyzed for chlorate content, each foot separately. It was found that most of what still remained undecomposed was concentrated in the third foot (see Table V). The season of 1931 was one of high rainfall. This possibly explains why growth starting on the plots later in the season appeared almost normal. The questions raised by these analyses are whether the chlorate will decompose as rapidly in the subsoil, and whether excessive evaporation later will bring it back close to the surface.

These plots were planted with wheat in 1932. All the plots germinated well and for the first part of the season no effect of the chlorate could be noticed. By August 1 some of the lower leaves were browned on Plot C. It appeared that there might be a slight reduction in yield on this plot. Plots A and B showed no apparent effect. This would indicate that not all of the chlorate has decomposed in the two years; but that it has leached quite far down into the subsoil.

A field series similar to the one above was treated with sodium chlorate in June 1931. Analysis for chlorate content made in August 1931 (see Table V) indicated a rapid leaching into the subsoil. In 1932 this series was divided lengthwise into four strips. The strips were sown with wheat, oats, barley, and flax, respectively. This was planned as a preliminary experiment to see if there was any difference in the resistance of various crops to chlorate toxicity. The yields are shown in Table IV.

The difference between check and Concentration C in the wheat strip is much less than the difference noted between similar concentrations the previous year. This is undoubtedly due to a greater rainfall and therefore a greater amount of leaching. The wheat and oat yields were each reduced

TABLE IV

YIELD OF GRAINS, 1932, ON PLOTS TREATED THE PREVIOUS YEAR WITH SODIUM CHLORATE

Crop	Concentration	Yield, per acre		Crop	Concentration	Yield, per acre	
		Grain, bu.	Straw, lb.			Grain, bu.	Straw, lb.
Wheat	Check	43.3	4850	Barley	Check	72.0	5728
	A	39.6	4330		A	30.6	3160
	B	25.2	2925		B	19.7	2208
	C	23.2	2497		C	10.4	1136
Oats	Check	104.5	6362	Flax	Check	6.1	5186
	A	90.9	4588		A	13.4	3724
	B	84.4	4257		B	13.4	3368
	C	62.3	3347		C	13.3	2464

about 40% by Concentration C of chlorate. The barley seemed least resistant, the yield being reduced about 85% by Concentration C of chlorate. The flax yield was not reduced. The increased yield of the treated plots over the check plot is due to weeds and volunteer grain being killed by the chlorate. It would appear that the chlorate had leached beyond reach of the more shallow-rooted flax plant.

Loomis, Bissey and Smith (11) state that chlorates are leached in the soil and under moist conditions become concentrated in the lower levels of the soil. This they say is next only to temperature in importance. Our investigations suggest that ordinary temperatures have little effect on the decomposition of sodium chlorate. Greenhouse temperature for 18 months has failed to decompose it completely in the soil. The following experiment suggests that the chlorate does not enter into chemical union with the soil, and that once leached out, the soil returns to its normal productive power.

On October 27, 1931, samples of the upper three feet were again taken (and analyzed for chlorate content). Portions of these samples were placed in tumblers in the greenhouse and seeded to wheat. Growth relations at the end of five weeks are shown in Table VI. This experiment indicates that the chlorate can be leached out beyond the limit of its toxic concentration and that a normal growth can be subsequently supported. Hulbert, Remsberg and Spence (9) state that cultivating the treated soil, before the following spring, renders the chlorate less effective. This, it is suggested, is related to the leaching factor. The surface soil can be leached of its chlorate beyond the limit of its toxic concentration. Cultivation would therefore permit the upper broken portions of the roots to produce healthy plants, away from the action of the chemical.

A series of three soils was placed in the greenhouse in 1930 and treated with sodium chlorate at the rates stated in Table I. The soils and the arrangement of crocks were the same as in the case of the sulphuric acid experiment previously discussed. The soils were maintained at optimum moisture and

cultivated every two weeks. Fungal and bacterial counts showed no definite trend. This was partly due to the fact that neither the temperature nor humidity could be controlled. An experiment discussed later, conducted in

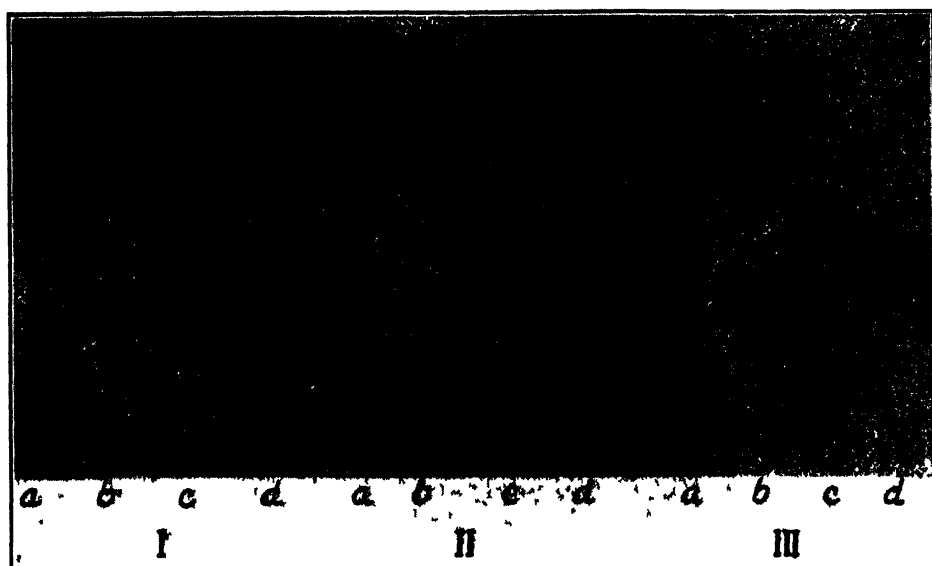


FIG. 3. Growth of wheat on soil maintained under greenhouse conditions six months after application of sodium chlorate I, Breton wooded soil. II, Edmonton black soil III, Winterburn fine sandy loam. a, check, b, $\frac{1}{4}$ lb. per 100 sq ft., c, $1\frac{1}{2}$ lb. per 100 sq ft., d, 3 lb. per 100 sq ft.

TABLE V

PENETRATION OF SODIUM CHLORATE IN THE EDMONTON SOIL UNDER FIELD CONDITIONS.
DETERMINATION MADE AUGUST 18, 1931

Concentration	Depth in feet	Applied July, 1930		Applied June, 1931		Amount added per plot
		Gm. per plot	Total per plot	Gm. per plot	Total per plot	
Check	0 to 1	0 0		0 0		
Check	1 to 2	0 0		0 0		
Check	2 to 3	0 02		0 02		
			02		02	None
Conc. A	0 to 1	0 0		0 0		
Conc. A.	1 to 2	123 0		82 0		
Conc. A.	2 to 3	240 0		369 0		
			363 0		451.0	742.0
Conc. B.	0 to 1	0 0		0 0		
Conc. B.	1 to 2	123 0		246 0		
Conc. B.	2 to 3	410 0		492 0		
			533.0		738 0	1483.0
Conc. C.	0 to 1	123 0		164 0		
Conc. C.	1 to 2	410 0		574 0		
Conc. C.	2 to 3	574 0		738 0		
			1107 0		1476 0	2966.0

NOTE: These determinations indicate that the sodium chlorate tends to leach down into the subsoil in a moist season.

TABLE VI

CROP (5 WEEKS GROWTH) ON FIELD SAMPLES TAKEN OCTOBER 27, 1931, FROM EDMONTON PLOTS PREVIOUSLY TREATED WITH SODIUM CHLORATE

Treated July	0-1 Foot	1-2 Feet	2-3 Feet
1930 Ch.	Normal growth	Normal growth	Normal growth
A	Normal growth	Normal growth	Normal growth
B	Normal growth	Normal growth	Some whitening
C	Normal growth	Some whitening, $\frac{3}{4}$ crop	Some whitening and retardation, $\frac{1}{2}$ crop
1931 Ch.	Normal growth	Normal growth	Normal growth
A	Normal growth	Some retardation, $\frac{3}{4}$ crop	Some retardation, $\frac{3}{4}$ crop
B	Normal growth	Some whitening, $\frac{3}{4}$ crop	Some retardation and whitening of leaves, $\frac{3}{4}$ crop
C	Some leaves dead, $\frac{3}{4}$ crop	Some whitening and retarding of crop, $\frac{1}{2}$ crop	Whitened and retarded, $\frac{1}{2}$ crop

NOTE: These results indicate that the sodium chlorate will leach down into the subsoil in a moist season. A normal growth is supported once the chlorate is leached out.

TABLE VII

SODIUM CHLORATE DECOMPOSITION IN POT CULTURES; GRAMS OF SODIUM CHLORATE PER 100 GM. OF SOIL

Soil	Series II	Series I	
	August, 1931	September, 1931	December, 1931
Black Ch.	—	—	—
Black A	.005	.001	—
Black B	.029	.019	.011
Black C	.080	.049	.051
F.S.L. Ch.	—	—	—
F.S.L. A	.005	.004	.006
F.S.L. B	.031	.010	.012
F.S.L. C	.050	.051	.040
Wooded Ch.	—	—	—
Wooded A	.016	.008	.011
Wooded B	.042	.020	.027
Wooded C	.086	.046	.036

NOTE: Series I was started in July 1930 in the greenhouse and maintained at optimum moisture throughout.

Series II was started in November 1930. It was left outside frozen till spring and then placed under greenhouse conditions in May 1931.

This table shows the more rapid decomposition of sodium chlorate under greenhouse conditions.

a controlled chamber, gave more significant results. At the end of six months the crocks were sown with wheat. The appearance of the crop is shown in Fig. 3. The heaviest concentration on the Breton grey soil practically prevented any growth whatever. Crops sown at 9 and 12 months showed a slight but steady improvement, especially in the Edmonton black soil. At the end of 12 months all the black-soil crocks were practically back to

normal excepting the C Concentration, which showed about a two-third's crop. At the end of the 12 months' period the C Concentration on the Breton grey still gave a 100% retardation of growth.

Table VII shows the amount of chlorates remaining in the soil. It shows that decomposition is taking place very slowly in the two poorer soils, and that in the richer black soil under greenhouse conditions it will take at least a year for 650 lb. per acre to decompose.

One year after the application of the chlorate one of each of the duplicates of this series was leached with six inches of tap water. The amounts of sodium chlorate obtained by this leaching are shown in Table VIII. The results indicate that it is possible to leach most of the chlorate out, but that it is more difficult to leach it out of the richer soils.

One month after these crocks were leached they were planted with wheat. As compared with the unleached crocks of the series, the leaching had removed much of the toxic element. However, the leached crocks all show some chlorate damage. In three weeks Concentration C wooded leached had a crop 3 in. high, whereas the wheat on the unleached did not appear through the surface (see Fig. 4).

There was practically no difference between Concentration C black soil leached and unleached. It was thought that possibly the chlorate might have left a toxic oxidation product in the soil of high organic matter content, but later experiments seem to disprove this. As suggested before, the chlorate is harder to leach out or extract from the richer soils. This suggests that chlorates remain relatively free in the soil and that they can be moved about in the soil by soil water.

A series of 24 crocks treated with chlorate was started November 10, 1930, and left outside, buried at ground level all winter. The frost did not appear to have any beneficial effect in aiding decomposition. Amounts of chlorate in this series are given in Table VII.

An experiment was outlined to test the neutralizing effect, if any, on chlorate, of farmyard manure, sulphuric acid and sulphur. Sixteen crocks of fine sandy loam were placed in the greenhouse. There were four each of (1) manure, 25 tons per acre; (2) sulphur, one ton per acre; (3) sulphuric acid, one ton per acre; and (4) four check crocks. Two crocks of each set were treated with sodium chlorate at the rate of 654 lb. per acre. They were kept fallow for six months, and at the end of this time a crop of wheat was planted. In general none of these satisfactorily neutralized the toxic effect of the chlorate. All samples treated with chlorate showed much

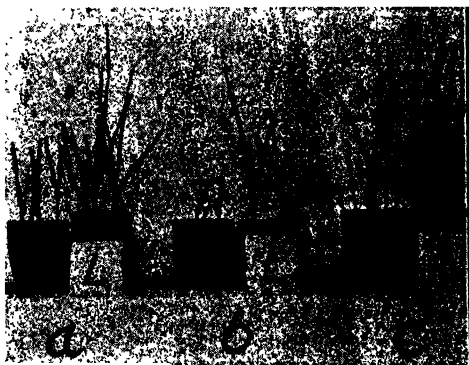


FIG. 4. The effect of leaching soil one year after treatment with sodium chlorate. Tumblers marked "L" were leached with six inches of water. a, fine sandy loam; b, wooded; c, black.

damage. There was no advantage whatever noted for the sulphur over the check. The sulphuric acid appeared slightly better than the check. The crocks treated with manure supported a growth twice as great as that of the check crocks, but it was badly whitened and of a spindly nature.

TABLE VIII

LEACHING OF CROCKS ONE YEAR AFTER TREATMENT WITH SODIUM CHLORATE.
FIGURES REPRESENT GRAMS OF SODIUM CHLORATE PER 3000 GM. OF SOIL

	First 2-in. leachings	Second 2-in. leachings	Third 2-in. leachings	Total 6 in. from 3000 gm.	Amount remaining after leaching	Amount decom- posed in one year	Amount applied
Wooded grey Ch.	.013	.006	.004	.023	.013	-.039	0.0
Wooded grey A	.469	.064	.022	.555	.053	.211	.78
Wooded grey B	.865	.186	.103	1.154	.079	.366	1.56
Wooded grey C	2.165	.386	.144	2.695	.106	.368	3.13
Fine Sandy Loam Ch.	.009	.007	.006	.022	.053	-.075	0.0
Fine Sandy Loam A	.525	.041	.034	.600	.053	.202	.78
Fine Sandy Loam B	.609	.148	.093	.850	.095	.690	1.56
Fine Sandy Loam C	1.000	.428	.137	1.565	.095	1.545	3.13
Edmonton Black Ch.	.009	.003	.003	.015	.039	-.054	0.0
Edmonton Black A	.463	.012	.007	.482	.039	.310	.78
Edmonton Black B	.542	.100	.048	.690	.119	.722	1.56
Edmonton Black C	1.173	.327	.180	1.680	.265	1.236	3.13

NOTE: The results indicate that it is possible to leach most of the chlorate out of soils, but that it is more difficult to leach it out of the richer soils. The quantities in the check samples represent the error of the determination, and allowance is made for this error in the column of amounts decomposed in one year.

The amounts of chlorates were determined after the crop was removed. Very little difference was noted in the results, although slightly more had decomposed under manure than in the other three, namely, sulphur, sulphuric acid and check crocks.

The above experiment suggested that if sufficient organic matter were added to the soil the effect of the chlorate could be neutralized. Further, that if an excess of easily decomposed alfalfa were added to the soil more definite information could be obtained as to the effect of sodium chlorate on nitrates and nitrification processes.

Three series of four jars each were prepared and treated with chlorate, at the same rates as in previous experiments. Series I was fine sandy loam alone, Series II was fine sandy loam plus 5% of alfalfa, and Series III was fine sandy loam plus 10% of alfalfa. Two weeks after application of the chlorate excessive decomposition was noted in Series III. The extent of decomposition was apparently proportional to the chlorate added.

Analyses for chlorate are given in Table IX. Where 10% of alfalfa was added practically all of the chlorate was decomposed within four weeks. In six weeks wheat was sown on a portion taken from each jar. Series I showed the expected harm from the chlorate. Series II showed a little effect of the chlorate and Concentration C supported about half a crop. In

TABLE IX

DECOMPOSITION OF SODIUM CHLORATE WITH EXCESS ORGANIC MATTER IN SOIL POT CULTURES; SODIUM CHLORATE IN GM. PER 100 GM. SOIL

		Added	4 Weeks	6 Weeks	8 Weeks	10 Weeks	14 Weeks
Series I Fine sandy loam	Ch.	None	—	—	—	—	—
	A	.026	.026	.014	.025	.019	.002
	B	.052	.037	.028	.037	.034	.033
	C	.104	.097	.083	.075	.080	.078
Series II F.S.L. + 5% of alfalfa	Ch.	None	—	—	—	—	—
	A	.026	.021	.012	.013	.011	.008
	B	.052	.037	.032	.023	.024	.031
	C	.104	.086	.067	.075	.070	.081
Series III F.S.L. + 10% of alfalfa	Ch.	None	—	—	—	—	—
	A	.026	.011	.010	—	—	—
	B	.052	.012	.011	.010	—	—
	C	.104	.006	.003	.009	.005	—

NOTE: These results indicate that sodium chlorate will decompose quite rapidly in a moist well-aerated soil, in the presence of 10% readily decomposable organic matter.

Series III a normal healthy crop grew on all four jars. There was no evidence of any residual effect from any of the concentrations. This suggests two conclusions: first, that it is possible to add enough organic matter to decompose the chlorate, and second, that when it has decomposed it does not leave any lethal oxidation products, and that the lethal substance is definitely the chlorate itself (see Fig. 5). This does not bear out Harper's (8) conclusion that chlorates disappear quickly from the soil in a warm moist climate and that their toxic effect is due to the depression of nitrating power or to the residual oxidation products of chlorate decomposition.

Nitrates were determined on the three series at intervals. Results are given in Table X.

On fine sandy loam without the addition of alfalfa, there was, if anything, a stimulation of nitrification up to six weeks due to the chlorate application. By eight weeks that difference was levelling out and by ten weeks a noticeable retarding of nitrate accumulation was noted. In Series II with 5% of alfalfa added a reverse effect was noted. After four weeks there was a rapid increase of nitrate in direct proportion to the chlorate added. This series is significant

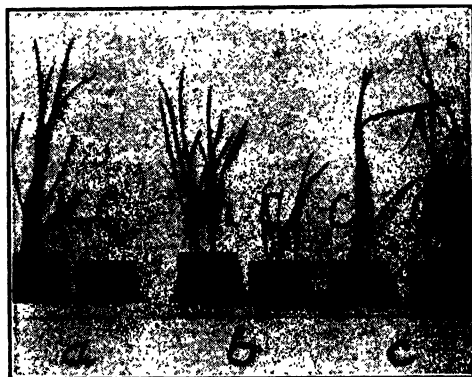


FIG. 5. The neutralizing effect of ground alfalfa added to sodium chlorate treated soil. Wheat was sown ten weeks after the chemical was applied. a, fine sandy loam alone; b, fine sandy loam plus 5% alfalfa; c, fine sandy loam plus 10% alfalfa.

TABLE X
NITRATE NITROGEN (P.P.M.) IN FINE SANDY LOAM POT CULTURES TREATED WITH
CHLORATE AND ALFALFA SEPT. 3, 1931

		2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	14 Weeks
Series I	Ch.	12	15	10	13	62	41
	A	12	15	10	22	45	8
	B	12	9	30	20	19	8
	C	14	18	40	23	10	11
Series II 5% alfalfa added	Ch.	25	45	50	80	51	72
	A	30	33	53	95	85	27
	B	27	30	110	210	146	72
	C	25	27	70	165	313	90
Series III 10% alfalfa added	Ch.	35	60	85	100	400	400
	A	36	15	75	146	311	200
	B	38	14	37	180	420	320
	C	40	12	32	130	473	300

NOTE: Series I indicates a small increase due to sodium chlorate followed by a similar depression.
Series II indicates that rapid nitrification of organic matter will occur in the presence of sodium chlorate, as much of the sodium chlorate remained undecomposed at 10 weeks.
In Series III practically all of the sodium chlorate was decomposed at 4 weeks.

as showing that nitrification will proceed in the presence of chlorate, because the chlorate had not decomposed. The depression of nitrates at four weeks in Series III is probably due to the excessive decomposition

of organic matter at that time. At this point practically all of the chlorate had decomposed and nitrification progressed rapidly after that date. Fig. 6 shows the progress of nitrification under Concentration C of each series.

To obtain more accurate information regarding the effect of chlorate on the microflora of the soil, four series were placed in the control chamber at 28° C. The soil used was Winterburn fine sandy loam, and each series consisted of a control and the A, B, and C applications of sodium chlorate. One series was removed for analyses every two weeks.

The amount of chlorate still obtainable in the soil at each analysis showed that although a little decomposition took place the greater part remained at the end of the two months (see Table XI).

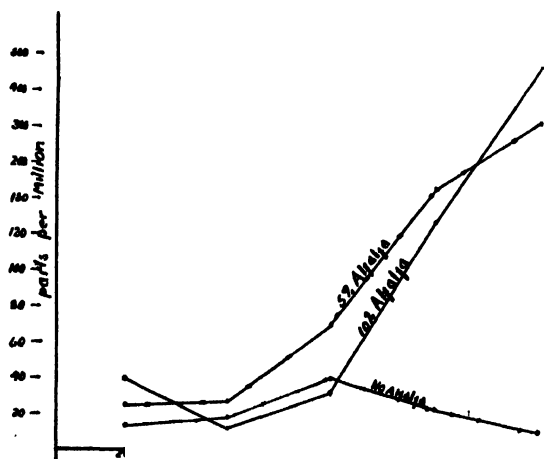


FIG. 6. Fluctuations of nitrates in soil with Concentration C of sodium chlorate added. This graph shows that nitrification will proceed in the presence of sodium chlorate under greenhouse conditions. With ground alfalfa added the nitrates increased.

There was a definite depression of numbers of fungi as shown by the plate count method. Their numbers show a gradual decline following the initial stimulation. This stimulation was probably due, at least in part, to the more ideal conditions of the experiment as compared to the previous condition of the soil. The counts at the end of the first two weeks show a depression in numbers relative to the concentration applied; the higher the concentration of chlorate, the lower the numbers.

TABLE XI

DECOMPOSITION OF SODIUM CHLORATE IN A CONTROLLED LABORATORY EXPERIMENT
USING FINE SANDY LOAM. TEMPERATURE, 28° C.

Applied May 5	May 19	June 1	June 16	June 18
None	.005	.006	.004	.002
.026	.0185	.018	.019	.016
.052	.043	.039	.039	.031
.104	.074	.076	.075	.060

NOTE: Multiplying quantities in the last four columns by $\frac{1}{4}$ will give approximate sodium chlorate left in the soil at each determination.

The total or bacterial counts were less regular than the counts of fungi but showed a definite decrease for the entire two months as a result of the sodium chlorate applications. Fig. 7 shows the above results graphically.

This experiment suggests that the application of sodium chlorate has a depressing effect on the microflora of the soil when readily decomposable organic matter is not also added to the soil. It is known that various chemical compounds have a decided effect on the behavior of micro-organisms. Such factors as flocculation, pleomorphism and the selective action of the media must be considered. No attempt was made to secure any data beyond the numbers as shown by the plate count. However, it is fully realized that this depression may be only apparent and that the results obtained may be due to factors other than a decrease in actual numbers.

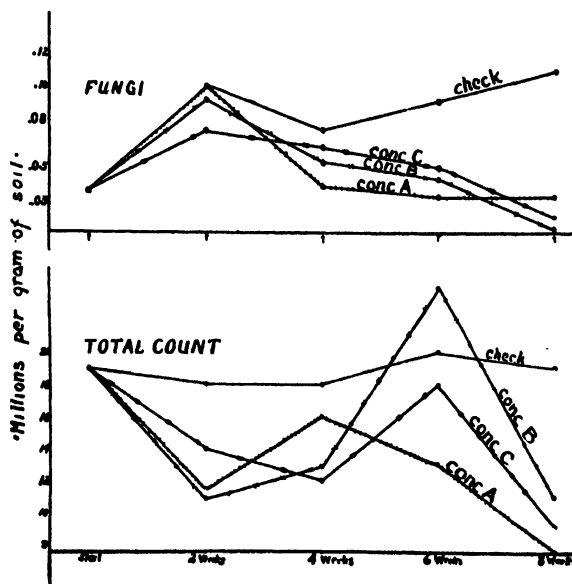


FIG. 7. Fluctuations in soil micro-organisms due to sodium chlorate treatment. This graph shows that under controlled conditions sodium chlorate depressed the numbers of soil micro-organisms.

Chemical weed killers have been quite extensively used in the irrigation districts of Alberta. In co-operation with the Field Crops Branch of the Provincial Department of Agriculture, samples were obtained from chlorate-treated plots at Brooks, Alberta.

As these plots had been subjected to both irrigation and seepage and since the chlorate was applied to the standing crop the chlorate analyses were very irregular. However, one or two things seem pertinent. The chlorate follows the water fairly consistently. In the seeped plots it is concentrated near the surface; in the irrigated plots it is concentrated down two or three feet. Plots receiving two irrigations after the chlorate treatment had the chlorate concentrated in the first and second foot. Those receiving three irrigations showed the highest concentrations in the second and third foot.

It is difficult to say if the chlorate carried down will decompose or if it will be carried back to the surface. It does seem reasonable to suggest that under ordinary irrigation practice shallow feeding plants might be grown the year following the application of the chemical, with reasonable success.

Sodium dichromate

As sodium dichromate was showing some promise as a herbicide in greenhouse experiments it was decided to include it in our list.

As a preliminary experiment, eight crocks of Edmonton black soil were treated in duplicate. The concentrations were similar in percentage to the chlorate concentrations used in previous experiments. After being kept moist in the greenhouse for four months these crocks were sown with wheat. At the end of one month no difference in growth whatever was seen in any of the crocks, *i.e.*, there was no residual lethal effect due to chromate. Determinations at the end of the five months showed that the chromate had all decomposed.

As a result of the above preliminary experiment a series of tumblers containing fine sandy loam soil was placed in a controlled chamber at 28° C. The series was increased from four to six. The concentrations used are given in Table I. A wider range was thought desirable because at present there is no definite knowledge as to its lethal concentration. The heaviest application also gives an oxidation equivalent to Concentration B of sodium chlorate.

One series was removed every two weeks. Numbers of fungi and bacteria were obtained and nitrates determined. The amount of Cr_2O_7 decomposition and the effect on a growing crop were also noted every two weeks. The effect on a growing crop was determined by planting and observing growth of wheat in small dishes of the incubated soil.

The experiment with sodium dichromate has given some significant results. Excepting in Concentration A the sodium dichromate had a depressing effect on nitrates and nitrification (see Fig. 8). The heavier applications reduced the nitrates to a very low amount. Concentration A acted as a stimulant and slightly increased the accumulation of nitrates over the check (see Table XII).

TABLE XII

AMOUNTS OF SODIUM DICHROMATE IN FINE SANDY LOAM TUMBLERS

	At start	2 Weeks	4 Weeks	6 Weeks	8 Weeks
Conc. Ch.	.0	.0	.0	.0	.0
Conc. A-	.013	.0	.0	.0	.0
Conc. A	.026	.011	.005	.007	.0
Conc. B	.052	.021	.011	.010	.006
Conc. C	.104	.064	.048	.025	.006
Conc. C+	.146	.117	.86	.030	.012

Amounts of nitrates in p.p.m. in tumblers of fine sandy loam treated with sodium dichromate

	9	10	17.5	15.4	15.0
Conc. Ch.	9	10	17.5	15.4	15.0
Conc. A-	9	3.5	12.5	20.0	25.0
Conc. A	9	3.0	5.1	3.0	1.5
Conc. B	9	3.1	5.1	2.0	1.0
Conc. C	9	5.0	4.4	1.6	2.0
Conc. C+	9	3.0	4.2	1.6	1.2

NOTE: These determinations indicate that sodium dichromate decomposes fairly rapidly in common soil, and that nitrification is greatly retarded in its presence.

The dichromate appeared to decompose under controlled conditions quite rapidly. By the end of the eight-week period only a trace could be recovered from the soil receiving the heaviest concentration, namely, C+ (see Table XII).

Sodium dichromate reduced the number of fungi for the period of eight weeks. Its action, however, on the total count appears to be selective. By comparison with the check plates there was a relative decrease in the main types of colonies with increasing concentration of chromate, but an increase in a typical pin-point colony.

In the soil receiving the higher concentrations the latter became very abundant, reaching the extent of over 100,000,000 per gram (see Table XIII).

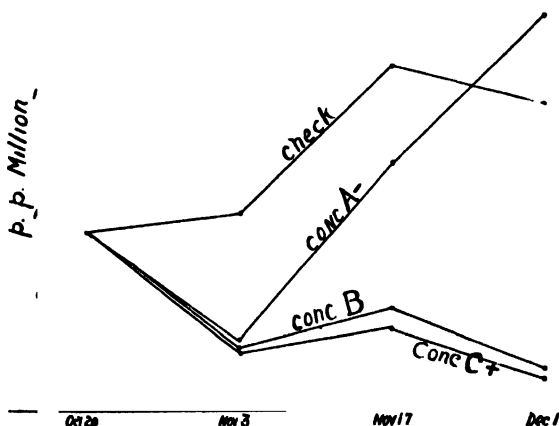


FIG. 8. Nitrate fluctuations with sodium dichromate treatment. This graph shows the depressing effect of sodium dichromate on nitrification. Determinations were made on fine sandy loam maintained under controlled conditions.

The growth of wheat every two weeks is shown in Fig. 9. A distinct retardation relative to the sodium dichromate was noted at first. However, later sets showed that this toxicity is not persistent and becomes less apparent. The retarded plants on the soil receiving the higher concentrations did not show the characteristic whitening of chlorate poisoning. Apart from their diminutive growth they appeared quite normal. This retardation persisted after the chromate appeared to have decomposed, which suggested that the effect may be due to factors other than oxidation or direct plant injury.

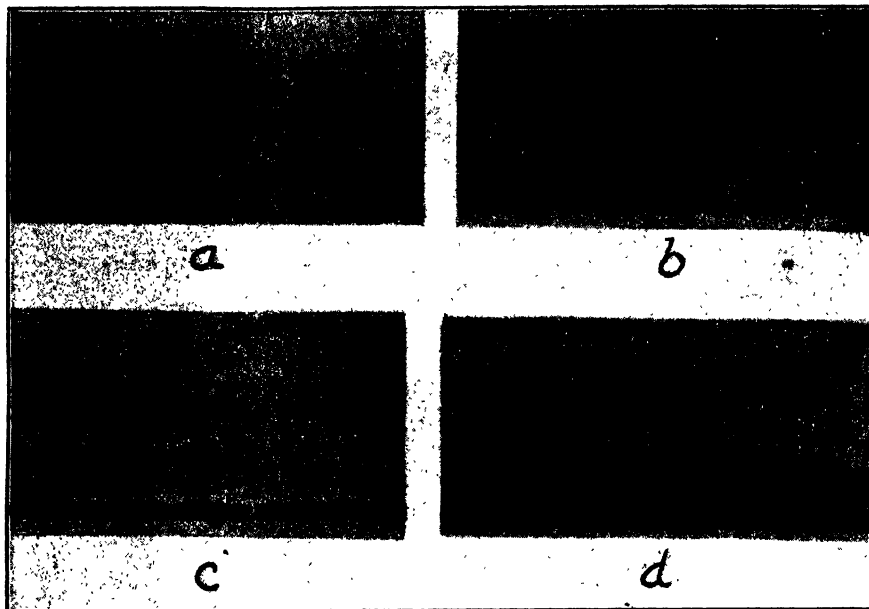


FIG. 9. Growth of wheat seedlings on fine sandy loam after treatment with sodium dichromate. Seed planted at various times after the chemical was applied. a, two weeks; b, four weeks; c, six weeks; d, eight weeks.

A series of fine sandy loam tumblers, check and Concentration C, was allowed to incubate in the control chamber for three weeks. At the end of this period they were removed and sown with wheat. One set of Concentration C was treated with potassium nitrate at the rate of 75 p.p.m. of nitrogen as nitrate nitrogen; a similar set was treated with nutrient solution at an equivalent rate with respect to nitrate nitrogen. It was thought that the depression of nitrates might have been partly responsible for the dwarfish growth supported by the chromate-treated soil. Neither the nutrient solution nor the nitrate application appeared to neutralize the effect of sodium dichromate.

One set of the check tumblers of fine sandy loam was treated with chrome alum. The amount of chromium added was equivalent to that in Concentration C of sodium dichromate treatment. The soil receiving the alum supported a normal crop. This indicates that the chromium ion in the cation form is not toxic to wheat plants in the concentration used.

TABLE XIII

FUNGAL NUMBERS IN TUMBLERS OF FINE SANDY LOAM TREATED WITH SODIUM DICHROMATE

Conc. Ch.	12	24	30	24	24
Conc. A -	12	15	20	23	20
Conc. A	12	11	24	19	24
Conc. B	12	18	21	27	23
Conc. C	12	16	11	19	13
Conc. C+	12	5	9	13	19

Times 1,000 = number per gram of soil.

Total count of bacterial numbers in tumblers of fine sandy loam treated with sodium dichromate

Conc. Ch.	15	13	44	36	35
Conc. A -	15	21	47	20	26
Conc. A	15	12	60	44	33 + 20*
Conc. B	15	7 + 10*	1	111	30 + 78*
Conc. C	15	3	8 + 70*	13 + 75*	8 + 111*
Conc. C+	15	2	5 + 65*	10 + 60*	12 + 101*

Times 1,000,000 = numbers per gram of soil.

* Very small colonies.

NOTE: These determinations indicate that the numbers of fungi and bacteria in soil are greatly reduced or affected by the presence of sodium dichromate.

It was found that 3% of alfalfa added, together with Concentration C of sodium dichromate, to fine sandy loam rapidly neutralized the toxic effect of sodium dichromate. The soil grew a normal crop when wheat was sown three weeks after treatment.

Sodium dichromate added to Edmonton black soil (which is much higher in organic matter content than the Winterburn fine sandy loam used) decomposed very rapidly. A series of tumblers of black loam was treated with Concentration C of sodium dichromate. Immediately after its application 96% could be extracted. In three days only 50% of the chromate could be recovered and in 15 days it had all decomposed.

TABLE XIV

AMOUNTS OF NITRATES IN P.P.M. IN FINE SANDY LOAM AND BLACK LOAM TREATED WITH CONCENTRATION C OF SODIUM DICHROMATE

	Start	8 Days	16 Days	25 Days	34 Days	43 Days
Winterburn F.S.L. Ch.	4	6	15	21	40	25
Winterburn F.S.L. Conc. C	4	Trace	Trace	Trace	4	Trace
Ed. Black Loam Ch.	20	34	80	105	150	100
Ed. Black Loam Conc. C	20	32	62	60	91	85

Sodium dichromate does not appear to depress the nitrates in the black loam to as great an extent as in the fine sandy loam. Table XIV shows the trend of nitrate accumulation over a period of six weeks in an incubation chamber of treated and untreated black loam and fine sandy loam. The nitrate content of the fine sandy loam treated with Concentration C was reduced to a trace for the entire six weeks' period. In the black loam the depression was only 15% for the same treatment at the end of the six weeks' period.

The above experiments have shown that sodium dichromate has an effect on the numbers and kinds of micro-organisms in the soil and that it depresses the amount of nitrates in the soil. This suggested that a measurement of the carbon dioxide evolution might furnish additional information regarding its effect on microbiological activity.

The apparatus set up to measure the carbon dioxide contained three units: one for untreated soil; one for chromate-treated soil; and a sand check. Two hundred grams of soil were used for each unit; one-half was placed in the bottom of a filtering flask and the other half in a connected separatory funnel over the flask. All soils were at optimum moisture when placed in the apparatus and the treated soil had Concentration C of sodium dichromate added. The air from the scrubbers, free from carbon dioxide, was drawn over the first portion of soil in the flask and then passed up through the other half in the separatory funnel. Bead towers containing carbonate-free sodium hydroxide collected the carbon dioxide given off by the soil. An eight-foot water head supplied the suction to draw the air through the apparatus. A flowmeter was inserted between the water head and the sodium hydroxide towers.

Each unit was aspirated 45 min. each day over a period of 45 days, at the rate of 12 litres per hr. Precautions were taken to prevent carbon dioxide contamination and a uniform check was obtained. The sodium hydroxide was titrated against standard hydrochloric acid using phenolphthalein for the first or bicarbonate end-point, and British Drug House indicator for the final end-point.

The results are shown graphically in Fig. 10. The large amount of carbon dioxide given off at the beginning is possibly due to the fact that the soil was air-dry for a considerable length of time before it was used in the experiment. The temperature was not controlled and this might explain some of the small daily fluctuations. There was a distinct retardation for the first two days in the carbon dioxide production from the soil treated with sodium dichromate. This gradually levelled out and from the 12th to 25th day the amount of carbon dioxide produced in the treated soil was greater than in the untreated soil. The curve would suggest something similar to a partial sterilization of the soil. After 12 days, when the amount of carbon dioxide produced by the treated soil became greater than in the untreated, practically all of the sodium dichromate would have been decomposed. These results seem to add more evidence to the fact that sodium dichromate acts quickly

and rather vigorously on the soil micro-organisms, but that the effect is not permanent. The speed with which the soil returns to normal is quite rapid especially if the soil is high in organic matter.

Experiments with sodium chlorate indicated that it was possible to leach the chlorate out of the soil beyond the limit of its toxic concentration. An analogous experiment was conducted using sodium dichromate. Nine leaching tubes, each containing 600 gm. of soil were used. There were three tubes of each of the three types of soil. To two tubes of each soil, Concentration C of sodium dichromate was added. Forty-eight hours were allowed, to bring the soil and solution to equilibrium. It was intended to leach six inches of water through each tube, but as there was still a considerable amount of chromate in the leachings with that amount, a total of ten inches was collected. There was only a trace of chromate in the fraction caught from a further ten- to twelve-inch leaching. The results are shown in Table XV.

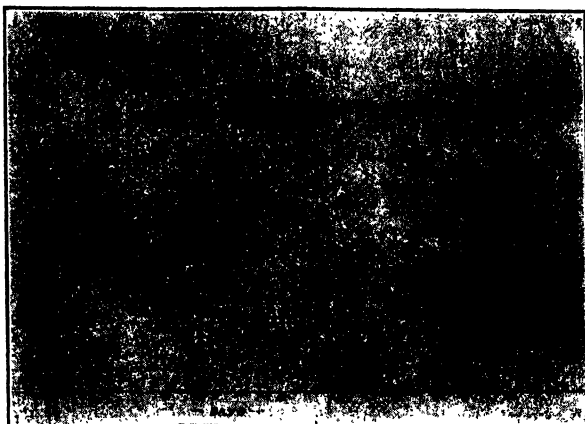


FIG. 10. This graph shows the effect of sodium dichromate on carbon dioxide production. The determination was made on Edmonton black loam.

TABLE XV

SODIUM DICHROMATE RECOVERED FROM SOILS BY LEACHING 48 HR. AFTER IT HAD BEEN APPLIED

Soil	Gm. of $\text{Na}_2\text{Cr}_2\text{O}_7$ added	Gm. of $\text{Na}_2\text{Cr}_2\text{O}_7$ retrieved	Av. gm. retrieved
Black 1	0.624	0.1001	0.1008
Black 2	0.624	0.1016	
Wooded 1	0.624	0.2146	0.2190
Wooded 2	0.624	0.2235	
F.S.L. 1	0.624	0.3608	0.3419
F.S.L. 2	0.624	0.3229	

The ten inches of water leached through contained 16.1% of the chromate that was added to the black soil; 35.1% of that added to the wooded grey soil; and 54.8% of that added to the fine sandy loam. This further suggests that the chromate is rapidly decomposed in the soil. If chromate is added to a soil and immediately extracted, from 92 to 96% is retrieved.

The soil from the leaching tubes was removed and planted with wheat. A normal crop grew on all soils. Or in other words no toxic effects appear to have remained in the soil.

A series of wheat plots in duplicate was sprayed with four concentrations of sodium dichromate on July 1. The concentrations used were one-half of those shown under Concentrations A, B, and C in Table I. Considerable damage resulted from the treatment. It killed the leaves of the wheat plant and blighted many of the heads. Concentration C reduced the yield of wheat to 4 bu. per acre as compared with 29 bu. on the check plot.

Water Culture Experiments

Experiments were conducted to determine the concentrations of certain chemical herbicides toxic to wheat plants in water or nutrient solution cultures. The effects of chemical herbicides upon plant roots may be modified by the presence of soil, and it was considered that the relative toxicity of different chemicals might be determined more definitely in a nutrient solution than in soils. There are fewer complicating factors in a nutrient solution, diffusion is rapid, and the solution can be standardized.

A standard plant culture solution, first recommended by Hoagland, was used in these experiments.

It was considered desirable first to test important perennial weed killers, since these are believed to have an important effect upon the roots of plants within the soil. The chemicals first tested were sodium chlorate and sodium dichromate.

TABLE XVI

EXPERIMENTS TO DETERMINE RELATIVE TOXICITY OF DIFFERENT CHEMICALS IN CONTACT WITH SPROUTING SEEDS

No. of gm. per litre	Solution	Wheat seeds set to sprout	Number germinated	Av. length of sprouts, in.	Variation in length of sprouts, in.
<i>Sodium chlorate</i>					
0.0	Dist. water	25	24	<i>Notes 4 days later</i>	
0.0	Nutr. sol.	25	24	1 3/4	1/2 - 2 1/4
1.0	Dist. water	25	25	2	1/2 - 2 3/4
1.0	Nutr. sol.	25	23	3/4	1/2 - 1 1/2
3.0	Dist. water	25	25	1 1/4	1/2 - 1 3/4
3.0	Nutr. sol.	25	23	5/8	1/2 - 1
				3/8	1/2 - 1 1/2
<i>Sod. dichromate</i>					
0.0	Dist. water	25	24	<i>Notes 5 days later</i>	
0.0	Nutr. sol.	25	24	1 1/2	1/2 - 2 1/4
1.0	Dist. water	25	24	2	1/2 - 2 3/4
1.0	Nutr. sol.	25	20	1/4	1/16 - 1/2
3.0	Dist. water	25	11	1/4	1/16 - 1/2
3.0	Nutr. sol.	25	7	1/8	1/16 - 1/4
				1/8	1/16 - 1/4

NOTE: Wheat seeds were sprouted between blotting papers in Petri dishes. After moistening with the original solution, moisture was maintained by adding distilled water.

The experiments indicate that sodium dichromate is much more toxic to germinating wheat than sodium chlorate, in equal concentration. A concentration of 1 gm. sodium dichromate per litre appears to be more toxic than a concentration of 3 gm. sodium chlorate per litre.

TABLE XVII
EXPERIMENTS TO DETERMINE RELATIVE TOXICITY OF DIFFERENT CHEMICALS IN NUTRIENT
SOLUTION CULTURES

Sodium chlorate, gm. per litre	Age of plants on adding chemicals, days	Notes 14 days later	Sodium dichromate, gm. per litre	Age of plants on adding chemicals, days	Notes 13 days later	Sodium chlorate, gm. per litre	Age of plants on adding chemicals, days	Notes 10 days later
0.0	18	Checks healthy	0.0	15	Checks healthy	0.0	32	Checks healthy
0.1	18	Like checks	0.1	15	Much wilted	0.1	32	Like checks
0.5	18	Partly wilted	0.3	15	Practically dead	0.3	32	Slightly wilted
1.0	18	Much wilted	0.5	15	Practically dead	0.5	32	Much wilted
3.0	18	Much wilted	1.0	15	Practically dead	1.0	32	Practically dead

NOTE: Duplicate litre (or quart) jars were used for each treatment, and 5 (usually) wheat seedling plants were set in each jar. Chemicals were added after plants had made a fair growth.

The experiments indicate that, when placed in contact with the roots, sodium dichromate is toxic to the wheat plants in much lower concentration than sodium chlorate; 0.1 gm. sodium dichromate per litre appears to be quite as toxic as 0.5 gm. sodium chlorate.

The relative toxicity of different chemicals in contact with sprouting seeds was determined by sprouting wheat seeds between blotting papers in Petri dishes. After moistening with the original solution, moisture was maintained by adding distilled water. Germination and growth of sprouts was observed four or five days after the seeds were moistened.

The experiment (see Table XVI) indicated that sodium dichromate is much more toxic to germinating wheat than sodium chlorate, in equal concentration. A concentration of one gram of sodium dichromate per litre appeared to be more toxic than a concentration of three grams of sodium chlorate per litre.

The relative toxicity of different chemicals in contact with plant roots was determined by adding chemicals to nutrient solutions in which plants were growing. Duplicate litre, or quart, jars of nutrient solution were used for each treatment and five (usually) wheat seedling plants were set in each jar. Chemical herbicides were added after plants had made a fair growth, and the effects, if any, on the appearance of the plants, were noted a few days later.

The experiment (see Table XVII) indicated that, when placed in contact with plant roots, sodium dichromate is toxic to the wheat plants in much lower concentration than sodium chlorate. For example, 0.1 gm. of the dichromate per litre appeared to be quite as toxic as 0.5 gm. of the chlorate.

Experiment with Barium Chlorate

A preliminary greenhouse experiment with barium chlorate was outlined. Duplicate crocks of fine sandy loam were treated with barium chlorate using the concentrations shown in Table I. The amounts used were equivalent in oxidizing values to the concentrations of sodium chlorate used throughout this work.

The effects on wheat sown six months after the application of the barium chlorate showed that it was at least as toxic as sodium chlorate.

Summary

1. Soil experiments with several chemical herbicides were conducted in the field, in the greenhouse and in a controlled chamber. Generally three typical Alberta soils were used for pot culture work.

2. The special methods adopted for the determination of small amounts of chlorates in soils and for the determination of nitrates in the presence of chlorates are outlined, and all methods used are stated in the body of the paper.

3. Sulphuric acid in quantities used for control of annual weeds appeared to have no residual lethal effect on the Alberta soils studied.

4. Copper sulphate in quantities used for the control of annual weeds appeared to have no lethal after-effect on these soils. Nitrification was but slightly affected following its application. It did increase the percentage of grain to straw when applied to the growing crop, and an appreciable decrease in yield due to the copper sulphate might have been shown if the check plots had not lodged.

5. Much of the sodium chlorate applied will remain undecomposed in moist soils under greenhouse conditions for at least 18 months.

6. Sodium chlorate retained its toxicity in the Edmonton field for at least one year.

7. Sodium chlorate will move with the soil water and in a period of average or high rainfall will concentrate in the subsoil, *i.e.*, leaching plays an important part in its distribution and toxicity.

8. With the rainfall of Edmonton, 1931 (approximately 17 in. from May to September inclusive) the surface foot was practically freed of the toxic effect of sodium chlorate. This suggests that shallow-rooted crops might sometimes be grown more successfully than deep-rooted crops following its application.

9. Sodium chlorate, in the quantities used in these experiments, did not prevent, or greatly affect nitrification. Nitrification proceeded rapidly in the presence of chlorate when nitrogenous organic matter was added to the soil.

10. In controlled laboratory experiments sodium chlorate had some depressing effect on the numbers of soil micro-organisms.

11. Twenty-five tons of manure, one ton sulphur or one ton sulphuric acid per acre in pot cultures did not aid decomposition of sodium chlorate in a marked degree.

12. Approximately 10% organic matter in the form of alfalfa in pot experiments promoted rapid decomposition of sodium chlorate. This suggests that under favorable conditions, the toxic effect of sodium chlorate may be removed from the surface soil by incorporating a large application of readily decomposable organic matter with the surface soil.

13. Sodium chlorate does not appear to leave any toxic oxidation products of its decomposition.

14. Sodium dichromate in comparison with sodium chlorate appears to decompose in the soil very rapidly. Practically all of it decomposed in eight weeks in Winterburn fine sandy loam and in two weeks in Edmonton black loam.

15. In controlled laboratory experiments sodium dichromate had a depressing effect on the numbers of soil micro-organisms. It also had a decidedly depressing effect on soil nitrification in pot cultures.

16. The addition of 75 p.p.m. of nitrate nitrogen to fine sandy loam three weeks after the application of sodium dichromate failed to neutralize the toxic effect of the chromate.

17. Fine sandy loam containing 3% of ground alfalfa supported a normal crop three weeks after a heavy application (Concentration C) of sodium dichromate.

18. Sodium dichromate reduced the amount of carbon dioxide produced from Edmonton black loam. The reduction was quite marked for the first two days.

19. Leaching ten inches of water through the soil, 48 hr. after the application of sodium dichromate, led to the recovery of only a small percentage of the chromate added. Wheat sown immediately after leaching, however, grew normally.

20. Water or nutrient solution culture experiments indicate that when placed in contact with germinating wheat seeds, and in contact with growing wheat plant roots, sodium dichromate is much more toxic to wheat than sodium chlorate in equal concentration.

21. A preliminary experiment indicates that the toxicity of barium chlorate is similar to that of sodium chlorate.

(NOTE:—In testing the residual effect on crop growth in all laboratory experiments, wheat was the only plant used.)

Acknowledgments

The authors wish to take this opportunity to express their appreciation to Dr. F. A. Wyatt, Head of the Department of Soils, for counsel and for criticism of the manuscript.

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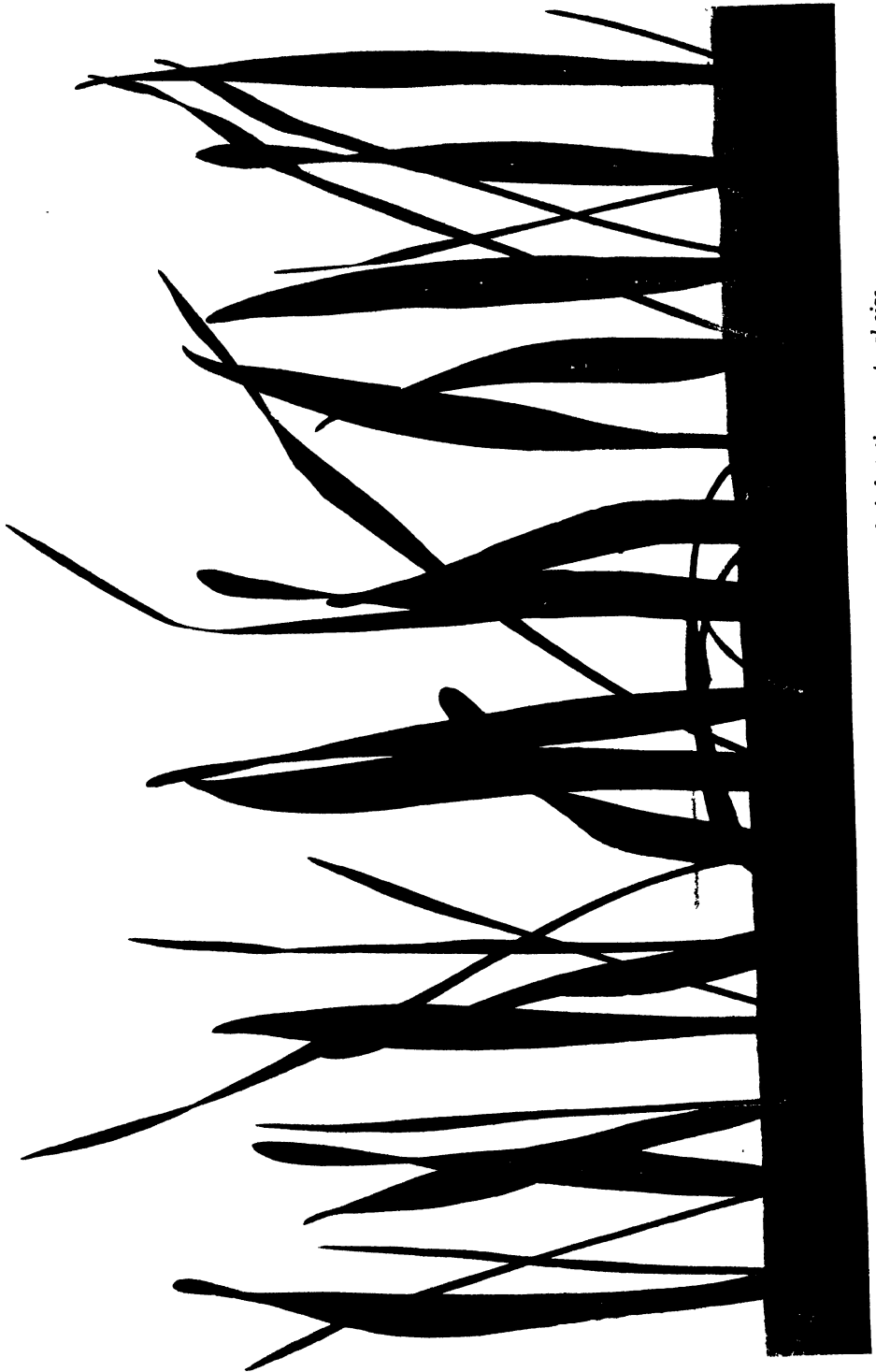


FIG. 1. Seedling barley plants showing the effect of nematode infestation—natural size

THE NEMATODE DISEASE OF NARCISSI AND CROP SEQUENCE¹

BY R. J. HASTINGS², J. E. BOSHER² AND W. NEWTON³

Abstract

The characteristic symptoms of nematode infection appeared in barley, oat and wheat seedlings as the sequence of planting the seed in soil infested with the nematode species, *Tylenchus dipsaci*, Kuhn, from narcissi bulbs. The severity of the infection was greater in barley and oats than in wheat.

In an investigation aimed at the establishment of a suitable crop sequence or rotation for the prevention and control of the nematode disease of narcissi, sterilized soil in six-inch pots was inoculated with the ground pulp of narcissus bulbs that was heavily infested with living nematodes of the bulb species *Tylenchus dipsaci* (Kuhn), and the pots were planted with barley, wheat and oats. Four weeks later, the characteristic symptoms of nematode infection, namely, the raised white spots, were apparent on the foliage of all three classes of seedlings. These raised white areas were more abundant along the midribs of the leaves than elsewhere. Although some distortion of the leaves occurred with all three classes of seedling, the distortion was conspicuous in the case of oats and barley. As judged by the foliage distortion and number of lesions, oats and barley are more susceptible to infection than wheat seedlings. No lesions were examined that did not contain living nemas and eggs. The appearance of nematode-infested barley seedlings is shown in Fig. 1.

The writers' observation of the severity of nematode infestation of barley seedlings does not support the conclusion of Massee (1, p. 548) that barley is a safe crop in a rotation. Massee pointed out that clover and oats are unsuitable rotation crops owing to the fact that the bulb nematode, *T. dipsaci* (syn. *T. devastatrix*) is responsible for clover sickness and the segging of oats.

Although barley, oats and wheat are included among the list of plants attacked by *Tylenchus dipsaci*, compiled by Steiner and Buhrer (2), no report of actual observation of barley and wheat infection by this nematode has been published by American or Canadian authorities. The writers' inoculation experiments show that under greenhouse conditions favoring rapid seedling growth, the characteristic raised yellow lesions appear less frequently than under slow growth conditions. This phenomenon may account for the lack of field observations upon wheat and barley infestations.

Acknowledgment

The authors wish to thank Dr. G. Steiner, United States Department of Agriculture, for confirming the identity of the nematode species.

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REVIEWS AND NOTES

THE NATURE OF THE MECHANISM OF POLYMERIZATION¹

BY HAROLD HIBBERT² AND STANLEY Z. PERRY³

During recent years considerable evidence has been obtained by Staudinger, Carothers, Haworth, Meyer and Mark, and others, indicating that highly polymerized products, such as cellulose, starch, rubber, etc., represent long-chain normal valence compounds formed from a simple building unit by addition of one molecule to another, although no conclusive proof of this "stepwise" formation has been provided.

In view of the close relation existing between polymerized ethylene oxide and cellulose, work was begun some eight years ago on the mechanism of polymerization of the former, and experimental evidence has now been obtained in support of the theory that this takes place in the following manner:—

A molecule of ethylene oxide is first activated by the catalyst (aqueous alkali). Ring fission then occurs followed by addition of water to give ethylene glycol. The latter, in turn, adds on to another molecule of the open chain form of ethylene oxide, giving diethylene glycol, and so on. The mechanism involved is the addition of HX to the open chain form of ethylene oxide, X representing $-(OCH_2CH_2)_yOH$, where y has the values of 0, 1, 2, 3, etc. The writers have isolated in this way the mono-, di-, tri-, tetra-, penta-, hexa-, hepta-, octa- and nona-ethylene glycols from such polymerization products, in addition to mixtures of the higher solid crystalline polyethylene glycols. Water is apparently necessary for this reaction, no change taking place with solid alkali alone. Substitution of a higher polyethylene glycol for the water yields a series of polyethylene glycols higher than the one employed.

Preliminary experimentation indicates that with methyl alcohol the terminal groups are (OCH_3) and (OH) , while with amines $(R.NH_2)$ they are probably $R.NH-$ and (OH) .

The evidence obtained is regarded as providing strong support for the view that in this, and a wide variety of other cases, the mechanism of polymerization consists, first, in the presence, or primary formation, of an unstable ring—the latter formed in many cases through the medium of the catalyst; second, in the transformation of this ring into an open chain form with activated terminal groupings followed by addition of the two addenda H and X , thus giving rise to chain formation.

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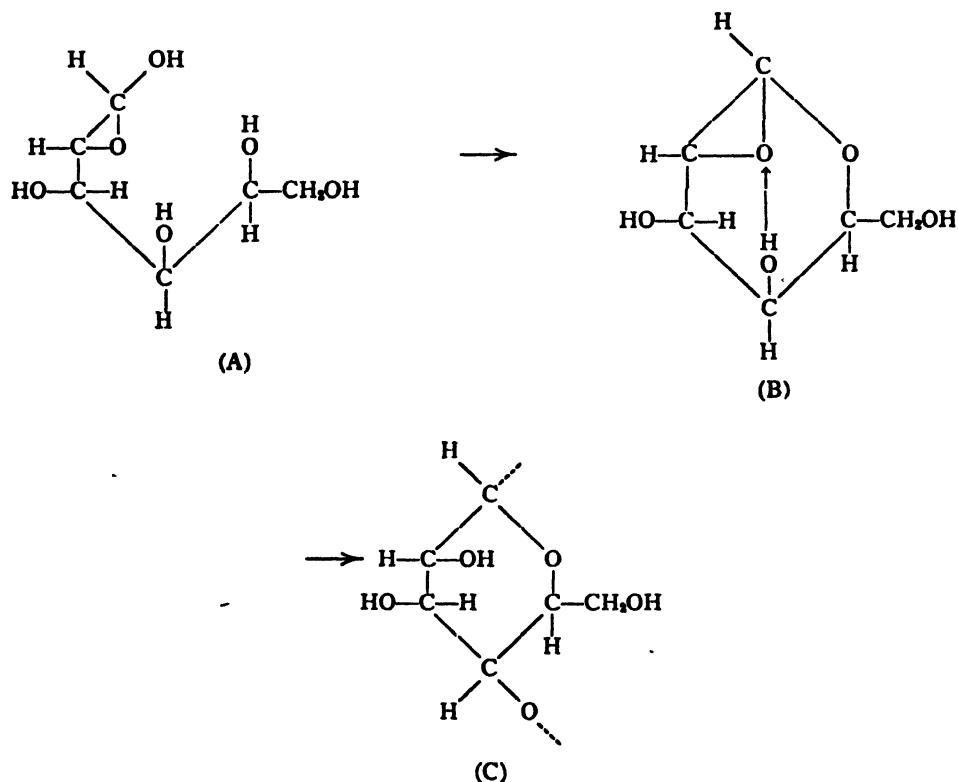
THE PLANT SYNTHESIS OF CARBOHYDRATES AND POLYSACCHARIDES¹

BY HAROLD HIBBERT²

In the preceding communication (1) attention is drawn to the experimental proof obtained recently regarding the mechanism of ethylene oxide polymerization.

The further suggestion is now advanced that this theory also provides a working hypothesis for the plant synthesis of the more complex sugars and polysaccharides.

It has been pointed out on several occasions in recent years, in addresses to the Cellulose Division of the American Chemical Society and elsewhere, that the synthesis of cellulose and starch in the plant probably takes place through the ethylene oxide equilibrium form of glucose and fructose (A) which, by loss of a molecule of water, may be assumed capable of yielding the glucose anhydride (B).



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Disaccharide formation can thus be regarded as taking place by the simple addition of a second molecule of a hexose (HX) to the free valence form of the ethylene oxide ring in (B), the disaccharide in turn then adding on a second molecule of (B) to give a trisaccharide, and the process representing an *intermolecular* chain synthesis.

On the other hand the synthesis of the more complex polysaccharide chains, such as cellulose, starch, glycogen, inulin, etc., may be visualized as being brought about by an *intramolecular* glycidol chain polymerization, as postulated in the conversion of (B) to (C). Such changes admit of an equally satisfactory explanation if considered as proceeding directly through the medium of the intermediate form (A) with simultaneous removal of water.

The theory thus represents an application of the chain mechanism for the thermal polymerization of glycidol first postulated by Nef (7, p. 232) and supported later by the work of Levene and Walti (2-6).

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SOME FEATURES OF ATMOSPHERIC EDDIES WITH APPLICATIONS TO THE VIBRATIONS OF TRANSMISSION LINES¹

By W. E. KNOWLES MIDDLETON²

Abstract

Some conclusions regarding atmospheric eddies, derived from the records of a pressure-tube anemograph at St. Hubert Airport near Montreal, are applied to the problem of the vibrations of transmission lines, furnishing a meteorological approach to the problem recently discussed by C. D. Niven.

It is found (1) that during glaze storms the wind at 60 ft. above ground is generally less gusty than the average; (2) that the mean duration of a gust varies inversely as the square root of the wind velocity, and is too large to resonate with the ordinarily observed period of "galloping"; and (3) that the steadiest winds invariably occur on nights of low wind velocity when a large negative lapse rate of temperature has been built up, leading to the small high-frequency vibrations due to eddies forming behind the cables.

The meteorological conditions accompanying a recent destructive case of "galloping" form the subject of part of the paper.

Introduction

C. D. Niven (8) has recently discussed from the experimental standpoint the problem of the various vibrations of transmission lines. This problem has of recent years proved of great practical importance to the engineering fraternity, especially where it concerns the vibrations of large amplitude, known as "galloping", "whipping", or "dancing". This part of the problem has been treated theoretically by DenHartog (4) and Varney (11), and from a general engineering standpoint by Archbold (1), Davison (2) and others. This paper is an attempt to contribute to the solution of the problem by setting forth some of the meteorological factors involved.

Data Available

The anemograph at St. Hubert Airport, near Montreal, gives a continuous record of wind velocity and direction at a height of 60 ft. above the ground. The normal record is on a time scale of 0.60 in. per hr., but a special quick-run drum is available, which has a time scale for the velocity of 7.2 in. per hr., making it possible to count the individual gusts. The normal records have been taken continuously from July 1930 until the present, and have

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yielded much information, shortly to be published, about the general *regime* of winds at St. Hubert. The quick-run records have been taken daily between 10h and 12h E.S.T. during the last four months.

Winds Associated with Glaze Storms

It has been suggested that "sleet storms"* may be associated with particularly gusty winds. As the gustiness of winds is largely influenced by convective processes, and as convection is greatest on bright sunshiny days, the suggestion seems *prima facie* unlikely. To test the matter, data for all the occasions during the winter of 1931-32 on which ice was reported to have formed on exposed objects at St. Hubert were examined, and the gustiness on each occasion compared with the mean gustiness for the same hour of the same month. The definition of gustiness adopted by the Meteorological Service of Canada is the one given by Dines (5), namely,

$$\text{Gustiness} = \frac{\text{max. vel.} - \text{min. vel.}}{\text{mean vel.}}$$

these quantities being taken over the entire hour.†

TABLE I
GUSTINESS IN GLAZE STORMS AT ST. HUBERT

Date	Hour ending	Gustiness		Remarks
		This hr.	Mean	
Dec. 11, 1931	16 h	0.80	0.82	
Jan. 17, 1932	14	0.72	0.85	
Feb. 11	14	1.40	1.06	One lull increased gustiness
Feb. 17	14	0.68	1.06	
Feb. 27	08	1.00	0.87	Anemograph doubtful
Mar. 22	14	0.25	0.53	
	16	0.33	0.52	
April 2	20	0.35	0.61	

It will be seen from Table I that only on two occasions was the gustiness during the glaze storm greater than the mean for the hour. Examination of the original records shows that on February 11 it was almost doubled by an isolated lull in the wind, and except for this would have been about 0.75. There is good reason to believe that on February 27 the anemograph was not functioning properly. In general, the results of this comparison support the inferences that would be drawn from general meteorological theory, which are further borne out by the large negative correlation coefficient (-0.65) at St. Hubert between mean monthly gustiness and mean monthly amount of cloud.

* The official meteorological definition of the word "sleet" on this continent is clear pellets of frozen rain. Ice accumulating on exposed objects is called "glaze". The engineering profession have in the past used "sleet" broadly to include this latter phenomenon, but the present tendency is to use the word "glaze" in conformity with North American meteorological usage.

† Since April 1932, the gustiness has been calculated from the records for ten minutes previous to each hour, instead of from the entire hour. This change does not affect the present results.

The gustiness calculated in this manner is only one of the many attributes of the wind; the above comparison by no means disposes of the possibility that the wind may have some peculiar feature during glaze storms, or even during some glaze storms and not others. More detailed examination of the wind structure is therefore necessary.

Duration of a Gust

It has been pointed out by Giblett and others (6) that the fluctuations in the direction and velocity of the wind are of three kinds. First there are the small rapid fluctuations with periods of the order of ten seconds, produced by the small eddies due to surface friction. Next, and superimposed on these, are the curious fluctuations with periods of the order of five minutes, for

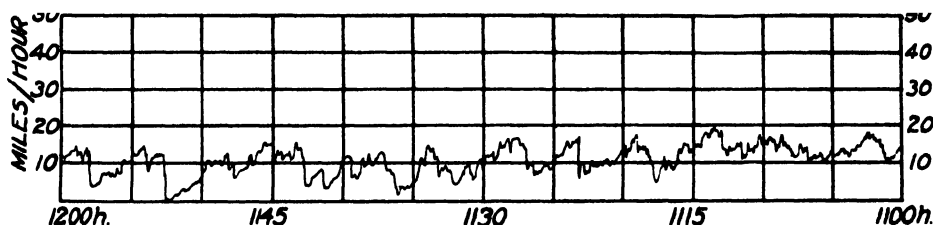


FIG. 1. Velocity of wind from 11h to 12h on May 10, 1932. Note that time scale runs from right to left.

which an ingenious theory has been proposed by Durst in the same paper (6). These two sets of fluctuations are well shown in the portion of the quick-run record of May 10, 1932, reproduced in Fig. 1. Thirdly, there are the major fluctuations of the order of a day or several days, due to the passage of barometric pressure-systems across the station. Of these three, only the first kind touches the present problem.

To arrive at the duration of a gust, or rather of a gust-and-lull, the quick-run records were divided into 5-min. intervals, and these into $2\frac{1}{2}$ -min. intervals. The number of peaks in the trace was counted for the first $2\frac{1}{2}$ -min. interval of each 5-min. interval, the results multiplied by 2, and the products averaged over an hour. The resulting number, divided into 300, gives the average period τ , in seconds, of a gust-and-lull considered as a unit. Furthermore, if this quantity is multiplied by the velocity of the wind in m. sec^{-1} , we obtain $2d$, twice the diameter of the eddies in metres. The necessity for the factor 2 will be made clear by a reference to Fig. 2, which must be understood as entirely diagrammatic. The actual structure of the wind is full of irregularities, well illustrated in the diagrams given by Sherlock and Stout (10).

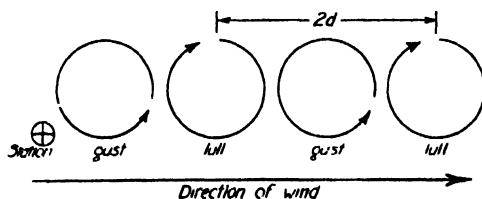


FIG. 2. Schematic diagram of gusts and lulls.

The results of these measurements are shown graphically in Fig. 3, in which the abscissas are wind velocities in miles hr^{-1} . As might be expected

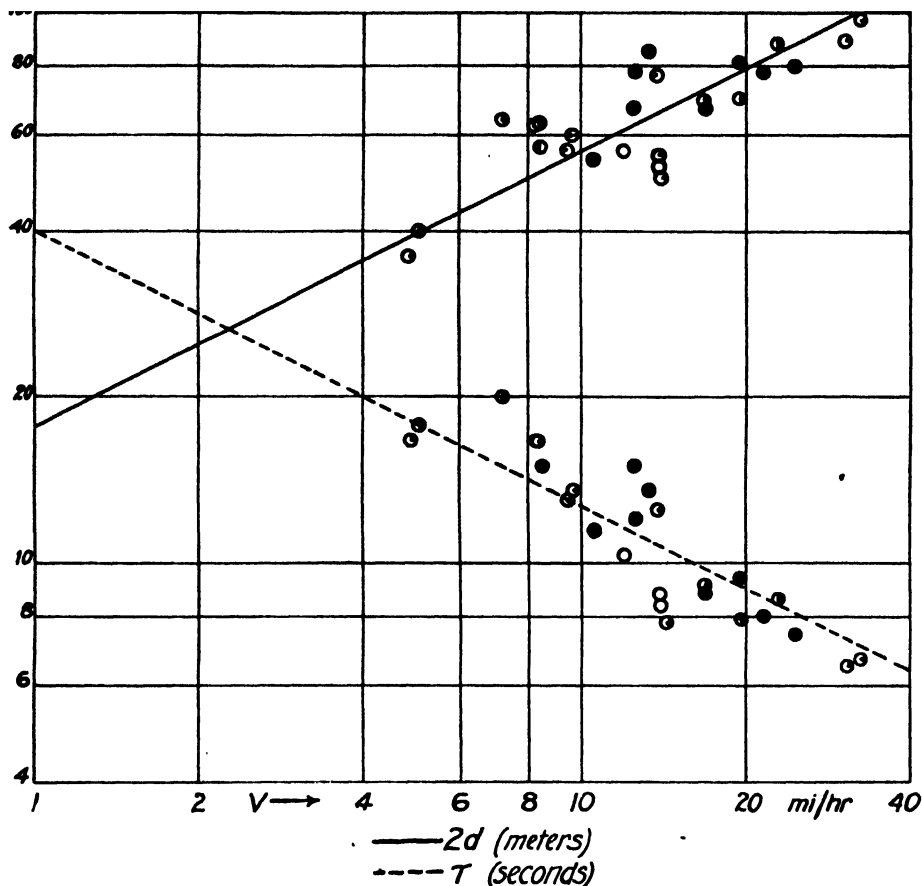


FIG. 3. Properties of gusts. Amount of black in small circles indicates amount of cloud.

from the nature of the data, the points show a good deal of scatter. However, the lines

$$\begin{aligned}\tau &= 40 V^{-0.5} \\ 2d &= 17.6 V^{0.5}\end{aligned}$$

represent the results fairly well.

We have now to inquire, what is the bearing of this on the problem of "galloping"?

The period of the vibrations known as "galloping" has been estimated at about 3 sec.; the excellent motion pictures of the phenomenon in the possession of the Hydro-electric Power Commission of Ontario, taken at Ellicott Creek near Buffalo during the glaze storm of Jan. 1, 1932, indicate that it is of this order. Now if we extrapolate the τ vs. V curve to $\tau = 3$ we get $V = 180$ miles hr^{-1} . No gusts with a period as low as 3 sec. or even 5 sec.

have been observed in this study. It is unlikely, therefore, that we are dealing with a resonance phenomenon; extremely unlikely, moreover, when we remember the completely irregular nature of the small variations in wind velocity. A similar irregularity manifests itself in the variations of the direction of the wind, and it has not been thought useful to attempt to discuss these quantitatively.

It may be objected that none of the records on which these figures are based have been obtained in glaze storms, an omission which will indeed be remedied at the first opportunity. However, a critical examination of the normal-speed records obtained under such conditions, as well as general meteorological considerations, suggest that especially small eddies in glaze storms are unlikely. In Fig. 3 the amount of cloud prevailing at the time is indicated by the amount of black in the small circles. It is evident that no difference in the effect of an overcast sky and of a clear sky is indicated by these results.

Meteorological Discussion of a Case of "Galloping"

The observations in the two preceding sections by no means close the question, and it is obviously necessary to adduce all possible evidence, and to test out all possible hypotheses, if the problem is to be finally solved. In pursuance of this idea it is considered desirable to set down here a discussion of the only reported case of "galloping" which has occurred sufficiently close to the instruments at St. Hubert to render such a discussion profitable.

Mr. J. W. Dunfield of the Southern Canada Power Company reported* a long-continued case of "galloping" on March 8, 1932, on the line between Farnham and St. John's, P.Q., which put the line out of service. This is a 48-kv. three-phase single circuit installation on two-pole H-frame wood construction, with suspension insulators. The direction of the line where "galloping" was observed is from east to west. The conductors are No. 00 stranded copper, span 400 ft., sag 7 ft., tension about 2000 lb., and spaced 7 ft. apart in a horizontal row. This line is only about 20 miles from St. Hubert. On this occasion heavy drifting snow was reported at St. Hubert, but not glaze. The temperature was 23.5° F. at 08h, having dropped fairly steadily from 32.5° F. at 20h the previous evening. It is thus rather likely that glaze had formed on the conductors during the night. The "galloping" was first observed at 08h, and "continued for several hours, and seemed to exist all along the line".

Fortunately a quick-run record of wind velocity is available for the period 10h to 12h on this date. Fig. 4 shows both the normal and quick-run records for March 7 and 8, and also a quick-run record for May 17, 1932, 10h to 12h, the latter being a normal wind of the same velocity as the one on March 8, and being inserted for comparison.

The reader will immediately notice in the quick-run records a difference in the *form* of the fluctuations. Those of March 7 and May 17 are usual, but those of March 8 are of a form which has not before been observed in these records. It is difficult to describe the forms of these curves, but

* *Private communication to Mr. A. E. Davison.*

attention may be drawn to the large *and sudden* increases and decreases in velocity on the 8th, often followed by comparatively steady velocity for 30 sec. or more. Before discussing these fluctuations we shall describe the general weather situation on this date.

Fig. 6 is a weather map for 08h on March 8, covering the eastern portion of the North American continent. The position of St. Hubert is indicated by two small concentric circles.

The controlling factor in the weather of the continent at this time was the great outbreak of polar air designated by P_0 , which had pushed southwards and eastwards for five days over the portion of the continent east of the Rockies. In addition, a depression originating on March 5 as a wave in a front over the Gulf of Mexico had developed rapidly (28.6 in. at Wilmington, N.C., at 08h on March 6) and passed up the Atlantic coast. On March 7 the centre was off Boston, and by the morning of March 8 was over Cape Breton Island.

The air masses connected with this depression were, besides the great polar outbreak behind it, a notable invasion of tropical air over the Atlantic, and a return movement of old continental polar air over Quebec. *On the morning of March 7 a somewhat diffuse cold front* had developed between this latter air mass and the main body of polar air, the difference of temperature across this front being about 10°F .

The southern portion of this front moved slowly eastwards, causing rain and snow in Michigan, Ohio, New York, and Quebec, and passed over St. Hubert at about 22h on March 7. It will be noted (see the top diagram of Fig. 4) that at this hour the wind completed a gradual change from N to SW. The thermogram, which is not reproduced, shows a sharp fall in the temperature at the same hour.

It was about eight hours after the passage of this front that the "galloping" was reported. The normal-run anemogram shows that at this time (08h March 8) the wind was 33 miles hr^{-1} from WSW, gustiness 0.7. At 10h the quick-run record was started, revealing the peculiar type of gustiness.

Two questions arise which must be settled as cases of "galloping", accompanied by appropriate meteorological records, become available. First, will winds of this sort cause "galloping", with or without ice on the cables; and second, are winds with this peculiar structure to be expected behind a front of the nature of the one which has been discussed? An anemometer with a very open time scale and small lag, such as has been described by Sherlock and Stout (9), would be of great value in this connection, especially if it could be set in motion immediately "galloping" is observed to occur.

The above case tends to support Niven's contention (8) that some peculiarity in the wind is responsible for the phenomenon. Many more cases must be studied, however, with sufficient observations available, before the real cause of "galloping" is determined.

* Readers unacquainted with the theory of fronts may find an excellent summary, written expressly for non-specialists, in Bulletin 79 of the National Academy of Sciences, National Research Council, Washington, 1931. (*Physics of the Earth—III. Meteorology.*)

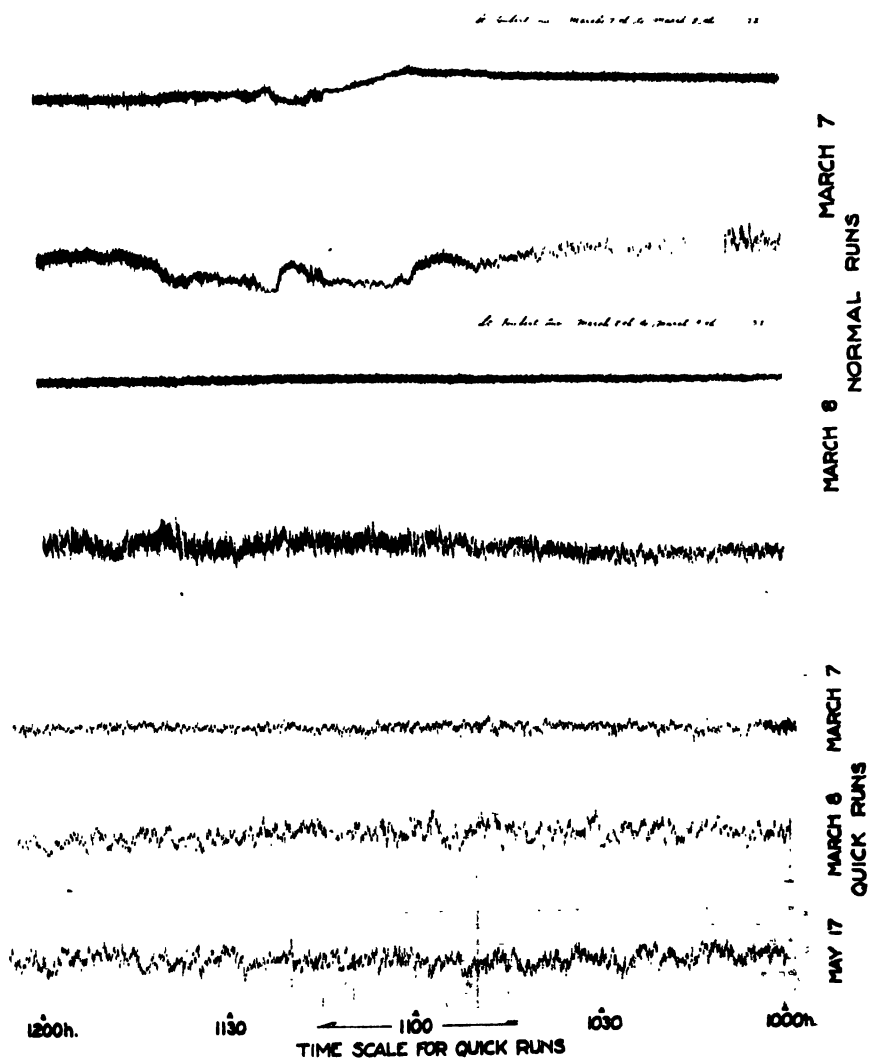


FIG. 4. Records of wind-structure.

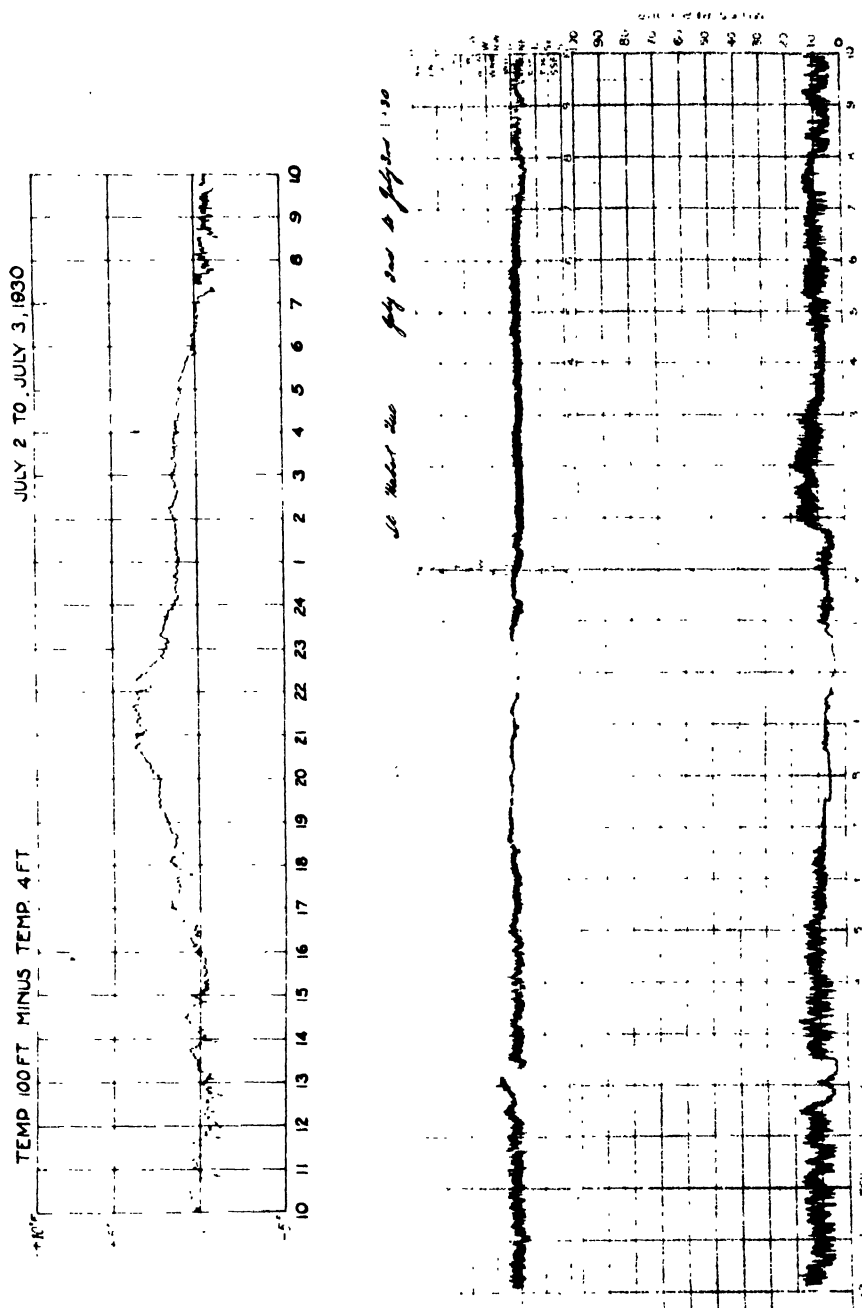
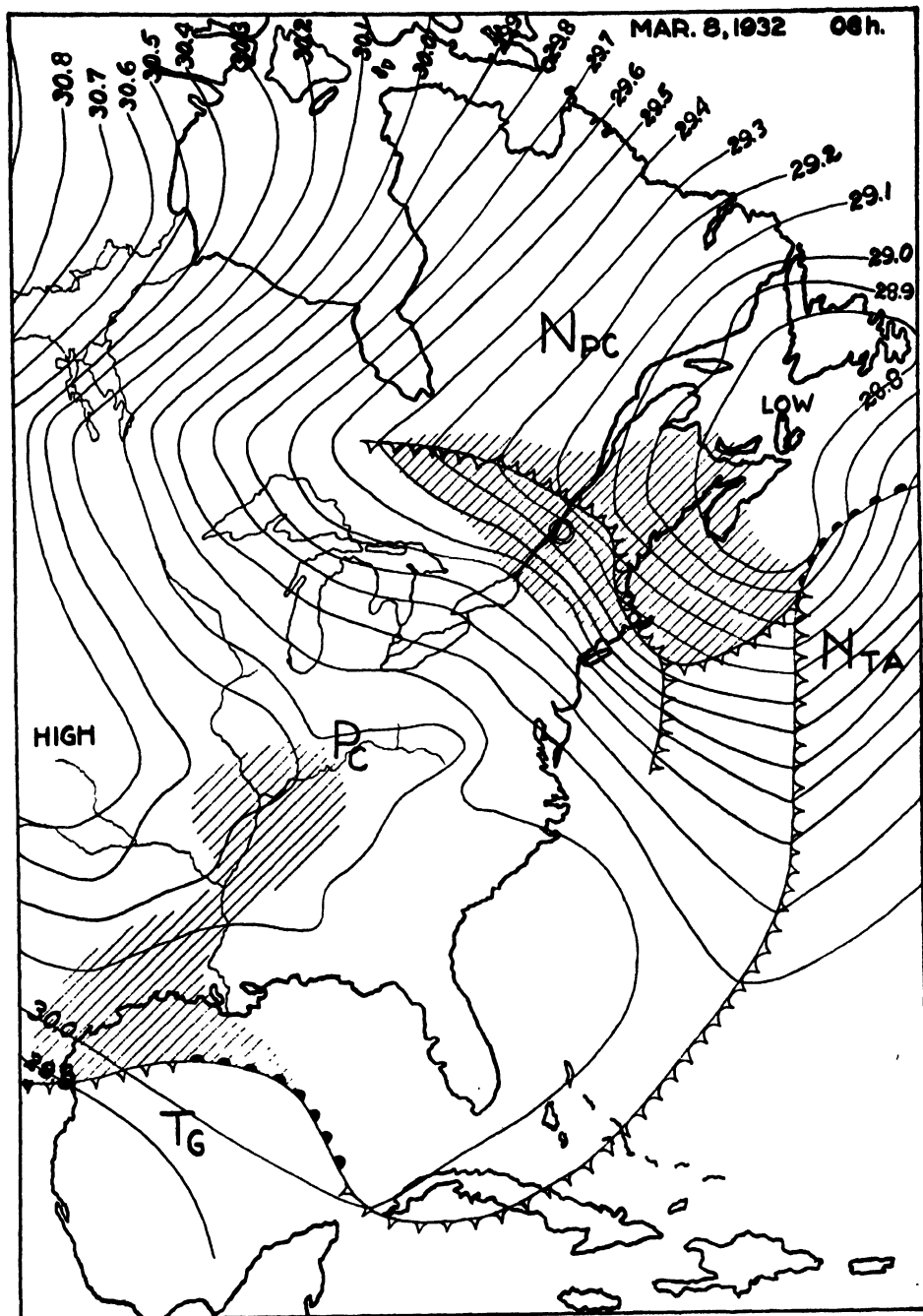


FIG. 5. Wind and temperature-gradient comparison.



LEGEND: ISOBARS AT 0.1-INCH INTERVALS —; COLD FRONTS ———; WARM FRONTS ———

FIG. 6. The hatched area denotes precipitation falling at 08h 75th meridian time.

The winds blow clockwise round the highs and counter-clockwise round the lows, following the isobars at about 500 m. above the surface, but tending into the lows and out of the highs at the surface. P_C , Continental (Canadian) polar air. N_{PC} , Returning continental polar air. T_G , Tropical gulf air. N_{TA} , Returning tropical Atlantic air.

Possible Effect of Ice on the Cable

The writer is indebted to Mr. J. Patterson for the suggestion that it may be possible that the fundamental cause of such large vibrations lies in a certain relation between the shape and size (in cross section) of the coating of ice on the conductors and the structure, velocity and direction of the wind at the time. DenHartog (4) and Davison (2) have shown that certain possible cross sections of ice, approximating to aerofoil sections, may give sufficient lift to account at least qualitatively for the upward movement of the wires. It would therefore seem desirable that if at all possible from a technical standpoint, measurements should be made of the ice coating when galloping occurs. In cases when the line has to be cut out of service this might not be impossible.

Unusually Steady Winds

Rapid vibrations of small amplitude, which lead to breaks in cable strands (3, 7), are due to eddies which form behind a cable (and also behind strings of insulators, tower members, etc.), in a steady wind. A given cable may vibrate in a number of configurations, but the essential factor is that the wind shall remain steady long enough for the vibration to be set up by resonance. In this connection the records shown in Fig. 5 will be of interest, as they are rather typical.

The lower portion of the figure shows the normal-speed direction and velocity records of the wind at St. Hubert from 10h July 2 to 10h July 3, 1930. The upper portion shows the difference in temperature between the top of a radio mast 100 ft. high and a screen at its base 4 ft. above the ground, over the same period, obtained by means of an automatically recording differential resistance thermometer. It will be noted that at about 14h on July 2, and again from 19h to 2245h, the wind became unusually steady. The upper record shows that at these times there was a considerable inversion of the lapse rate of temperature, which means that at these times the lower layers of the atmosphere were extremely stable, a condition unfavorable for the formation of the short-period gusts and absolutely prohibiting (according to Durst's theory referred to above) the presence of the longer ones. A wind as steady as is indicated at 20h might easily lead to short-period vibrations in a line. Such conditions often occur over flat country, especially at night.

Such steady winds are invariably very light winds. Niven tested wires in a wind tunnel with velocities ranging from 20 to 60 m.p.h. (8). As no natural wind of this speed could possibly be steady, at least over land, it would have been interesting to try velocities of the order of five miles per hour, to approximate actual conditions.

Conclusion

The writer would be glad to investigate, as far as available records allow, the meteorological conditions accompanying observed cases of "galloping" and of excessive short-period vibration. Only in this way can a proper

meteorological approach to the problem be made. The phenomenon of "galloping" especially is so infrequently observed that it will probably take many years before a satisfactory statistical treatment can be applied to the meteorological conditions which accompany it. The whole problem is obviously one which calls for the co-operation of the engineer, the physicist, and the meteorologist.

Acknowledgment

It is desired to acknowledge the kindness of Mr. A. E. Davison of the Hydro-electric Power Commission of Ontario in placing at the disposal of the writer a large file relating to the subject.

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NOTE ON SOLID-LIQUID EQUILIBRIA IN SOME TWO-COMPONENT SYSTEMS INVOLVING HYDROGEN CYANIDE¹

By A. L. PEIKER² AND C. C. COFFIN³

Abstract

The freezing-point curves of the two-component systems hydrogen cyanide-water, hydrogen cyanide-formic acid, hydrogen cyanide-formamide and hydrogen cyanide-benzaldehyde have been determined. These systems are all of the simple eutectic type and show no evidence of molecular compound formation. A chemical reaction prohibited the investigation of solid-liquid equilibria in the case of halogen hydride-hydrogen cyanide systems.

Introduction

Incidental to an investigation of the effect of molecular compound formation on the velocity of certain reactions involving hydrogen cyanide, an attempt was made to determine the freezing-point curves of mixtures of hydrogen cyanide with water, hydrogen chloride, hydrogen bromide, benzaldehyde, formic acid and formamide. A chemical reaction in the liquid state at low temperatures was found to prohibit the study of solid-liquid equilibria in the halogen hydride-hydrogen cyanide mixtures. The data obtained for the other systems are here presented.

When this work was undertaken the only data on the freezing points of hydrogen cyanide-water systems were those of Gautier (3) and Lespieau (4). The work of the former was carried out before the formulation of the phase rule; the temperatures observed were not those of phase equilibrium and hence were valueless for the purpose in hand. The latter was concerned with concentrations of water in hydrogen cyanide up to only about one mole per cent. Quite recently however Coates and Hartshorne (1) published an account of a complete and apparently very careful study of the system, so that this part of the present paper would be superfluous were it not for the fact that the close agreement between the results of the two very different experimental procedures affords excellent confirmation of the essential reliability of each. While the method to be described is not capable of such accuracy as the heating- and cooling-curve method of Coates and Hartshorne, it nevertheless possesses certain advantages of speed and convenience in manipulation and has proved to be very suitable for handling such a toxic substance as hydrogen cyanide.

¹ Manuscript received January 9, 1933.

The work described in this paper was carried out during the Spring of 1929 under the direction of Prof. O. Maass in the Physical Chemistry Laboratory, McGill University, Montreal, Canada.

² Instructor in Chemistry, Trinity College, Hartford, Conn.; at the time graduate assistant in Chemistry, McGill University.

³ Associate Professor of Chemistry, Dalhousie University, Halifax, Nova Scotia, Canada; at the time holder of a fellowship under the National Research Council of Canada.

Experimental

The hydrogen cyanide, prepared as described in "Organic Syntheses", was passed through four calcium chloride tubes immersed in a water bath at about 40° C. and then into the apparatus of Fig. 1 at *N*. It was condensed in the bulb *K* which was surrounded with ether and solid carbon dioxide. When a quantity sufficient for the work in hand had collected in *K*, the generator was shut down and vented and the system to the right of the gas burette *B* was evacuated through *L* by a Hyvac pump on a balcony outside the building. (All traps, manometers, etc., e.g., at *O* in Fig. 1, were glass-sealed to a common vent line leading to the outside atmosphere.) The

hydrogen cyanide was dried by distilling it back and forth several times through the phosphorus pentoxide tube connecting bulbs *K* and *H*. The middle third was then distilled to the storage bulb *G* and the tap *J* was closed. The vapor pressure measured on the manometer *M* was 27.0 cm. at 0° C. In Landolt-Bornstein Tabellen the value given is 26.8 cm. No noticeable

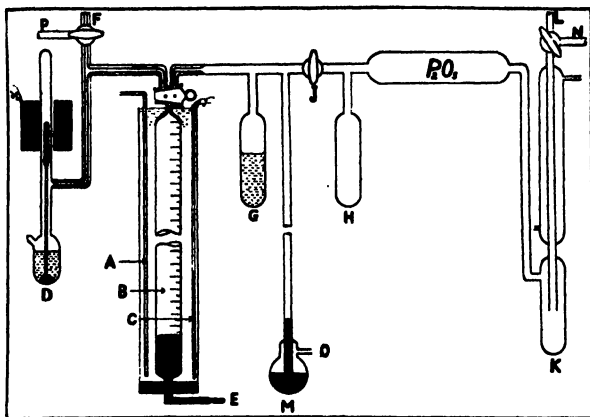


FIG. 1. Diagram of apparatus.

change in the vapor pressure or color of the liquid had occurred after it had remained three months in *G* at room temperature and about one-third of the original quantity had been removed for experiments. It is thus evident that no appreciable polymerization had occurred in the dried and purified liquid. The tubing of the generator system and of all the apparatus to the right of the phosphorus pentoxide tube was covered in a few days with the brown polymer of hydrogen cyanide.

The method of making up mixtures of known composition and determining their melting points was as follows. A weighed amount of distilled water was transferred from a pycnometer to the thin-walled bulb *D* (capacity about 5 cc.) through the side arm which was at once sealed off. The water was then frozen and kept at -78° C. while the system was evacuated through *F*. Three fusions and crystallizations *in vacuo* were usually required to remove all the dissolved air. By manipulating the stopcock and the levelling bulb (attached at *E*) of the gas burette, *B*, a known amount of hydrogen cyanide was transferred from *G* to *D* in a manner that is evident from the diagram. In order to keep the hydrogen cyanide in the gaseous state at a known temperature while its volume was being measured, the gas burette was surrounded with a large water jacket provided with an electric heater, *C*, and a tube, *A*, for air stirring. The temperature of the water was usually kept at about 35° C.

When the hydrogen cyanide had been added and the burette tap closed, the magnetic stirrer in *D* was started and the bulb was immersed in a large transparent Dewar flask filled with an alcohol-ether mixture and stirred by a current of dry air. The bath was cooled by adding pieces of carbon dioxide snow or "dry ice" until the solution in *D* froze, stopping the stirrer. The bath was then allowed to warm fairly rapidly until the contents of the bulb had melted sufficiently to allow the stirrer to function. At this point the bath was again brought under control and the rate of temperature rise regulated to about 0.1°C. in two or three minutes. This could easily be effected after some practice by adding powdered "dry ice" or increasing the stream of air through the bath. The thermometer in the bath and the vigorously stirred contents of *D* were kept under continual observation—temperatures and estimates of the amount melted being noted from time to time. When the crystals had almost disappeared the bath was slowly cooled again until the quantity of crystals had definitely increased. This was repeated several times for each mixture and the mean of the upper and lower temperature limits (usually differing by less than 0.2°C.) was taken as the melting point. Another addition of hydrogen cyanide was then made and the process repeated.

Temperatures below -30°C. were measured on a platinum resistance thermometer and above -30°C. on a mercury thermometer graduated in 0.2°C. and calibrated against a Reichenstalt standard.

The greatest source of error in such a method probably lies in the fact that the temperature of the bulb must lag behind that of the bath. However by allowing the bath temperature to change very slowly and at as nearly as possible the same rate during heating and cooling, as well as by using thin glass bulbs and small quantities of material, it is to be expected that such errors may be practically eliminated.

By connecting *D* to the rest of the apparatus with short lengths of capillary tubing, the volume of the vapor phase was kept small and any errors arising from this source were negligible.

The overlapping portions of the curves obtained in different runs always coincided, so that although the composition errors are necessarily cumulative during a run they are evidently quite small.

Results

The System Hydrogen Cyanide—Water

The results obtained for the hydrogen cyanide-water mixtures are plotted as circles in Fig. 2. It will be noted that within the probable limit of error the majority of the points fall upon the smooth curve which is drawn through the data of Coates and Hartshorne.

As these authors point out, the system is of the simple eutectic type and shows a marked inflection at about 35 mole per cent of hydrogen cyanide. On the water side of the diagram the curve is linear up to about 9 mole per

cent of hydrogen cyanide, which is unassociated at these concentrations. After this point, however, the apparent degree of association increases with increasing hydrogen cyanide content until at 35 mole per cent and -16.0°C . a close approach to complete association or non-solubility (*i.e.*, separation of another liquid phase) is indicated. By supercooling, Coates and Hartshorne actually found such a metastable two-liquid phase equilibrium for which they give the critical solution temperature as -24.0°C . and the critical composition as 35 mole per cent of hydrogen cyanide.

The slope of the curve on the hydrogen cyanide side shows that in dilute (less than about one mole per cent) solutions water is unassociated in hydrogen cyanide. The apparent degree of association increases continuously as the eutectic is approached.

The change in the appearance of the crystals with increasing hydrogen cyanide content may be roughly summarized as follows. From 0 to 20% of hydrogen cyanide—plate-like crystals (ice); from 50 to 60%—very small crystals (ice); from 60 to 80%—mixture of small crystals (ice) and long needles (hydrogen cyanide); from 80 to 100%—long needles (hydrogen cyanide). For some distance on either side of the eutectic the melting points of each crystal form could be noted. The two metastable solids occasionally observed by Coates and Hartshorne escaped notice.

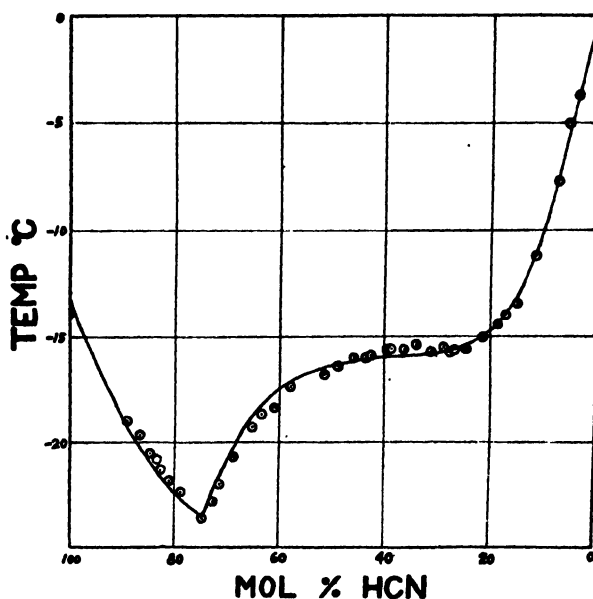


FIG. 2. Melting points of hydrogen cyanide-water mixtures.

The Systems Halogen Hydride—Hydrogen Cyanide

An attempt was made in the case of the halogen hydride—hydrogen cyanide mixtures to utilize the method described above with the modification that known amounts of pure dry hydrogen chloride or hydrogen bromide were condensed in *D* through the tube *P* by means of liquid air. It was found however that a chemical reaction which gave a white sandy precipitate took place in the liquid state at a velocity sufficient to render such a method impossible. In the hope that this reaction could be inhibited by keeping the mixtures frozen until the melting point could be quickly determined, recourse was had to a "bulb" method (2). Known amounts of the gases were condensed by means of liquid air in small glass bulbs which were then sealed

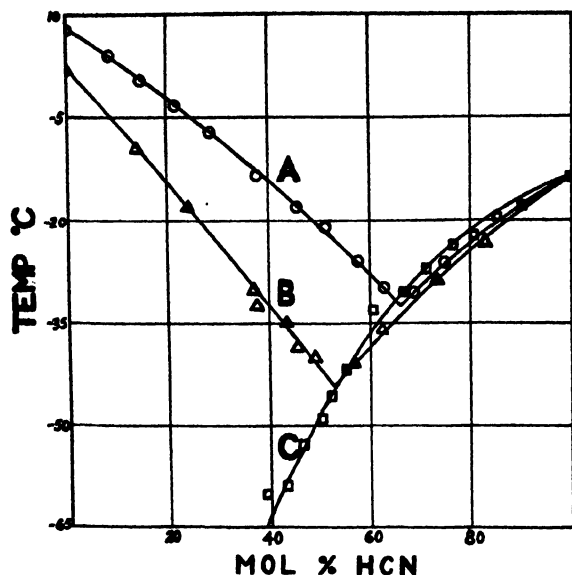


FIG. 3. *Melting points of mixtures of hydrogen cyanide and (A) formic acid, (B) formamide, (C) benzaldehyde.*

gave them the formulas $\text{HCl} \cdot \text{HCN}$ and $3\text{HBr} \cdot 2\text{HCN}$.

The Systems Benzaldehyde-Hydrogen Cyanide, Formic Acid-Hydrogen Cyanide and Formamide-Hydrogen Cyanide

The experimental method in these cases was similar to that used for the water-hydrogen cyanide system. The results are plotted in Fig. 3. As is apparent from the curves each of the three systems is of the simple eutectic type showing no indication of molecular compound formation. Indeed the opposite tendency is evident—self-association characterizing every branch of the curves. On the hydrogen cyanide side of the diagram the molecular weights calculated from the slopes of the first half of the curves show that each solute exists as approximately double molecules. The slopes of the branches on the other side of the diagram indicate a considerable and fairly constant association.

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off and kept at -190°C . until all was in readiness for the melting point determination. This method was unsuccessful, however, as the reaction seemed to take place to a considerable extent during the preliminary melting necessary to mix the contents of the bulbs. This was particularly true of the hydrogen bromide mixtures which reacted violently and several times exploded as they melted. The solid reaction product, while always white in the case of hydrogen chloride, varied from a light pink to a deep red color in the hydrogen bromide reactions. These compounds were presumably those prepared and analyzed by Gautier (3) who

THE SULPHUR CONTENT OF CRUDE NAPHTHA FROM TURNER VALLEY IN RELATION TO REFINING PRACTICE¹

By J. W. SHIPLEY²

Abstract

Typical samples of Turner Valley naphtha were examined for their sulphur content and for their behavior towards common refining treatments. The sulphur content ranged from 0.15 to 0.19% and consisted of sulphides, organic disulphides and mercaptans. Thiophenes were present, but in very small amounts. No free sulphur or peroxides were detected. Distillation concentrated the color and color producing compounds and the gum and gum producing compounds in a small high-boiling residual fraction. Exposure to light, even in the absence of air, led to the development of a yellow color and to gum formation in all undistilled samples, treated or untreated. Distillation up to 165° C. yielded a fraction (95% by volume) which remained colorless and free from gum formation when exposed to the air and to light for over eight months.

Recovery of the propane and butane is recommended, and the treatment of the crude naphthas with sodium hydroxide solution or with sodium hydroxide and sodium sulphide solutions followed by distillation is suggested as a means of producing a fairly satisfactory gasoline. The caustic wash alone reduces the sulphur content to about 0.1%, while the alkaline-sodium sulphide wash reduces it to about 0.05%.

Sulphuric acid, sodium hypochlorite, caustic wash, sodium plumbite and cupric chloride treatments were examined and the refined products compared for sulphur content, sweetness, corrosion, gum formation and color. None of these treatments prevented gum formation or the development of color when the treated samples were exposed to light.

Introduction

Crude naphtha is separated from the Turner Valley (45 miles southwest of Calgary, Alberta) wet gases by permitting the casinghead gas to expand from the well pressures to a pressure of 200–250 lb. in baffled metal separators. The drop in pressure, with consequent expansion and cooling, causes the separation of some of the more condensable hydrocarbons in liquid form. This liquid coming from the separators forms the principal source of motor fuel from the Turner Valley wells. The yield of liquid naphtha varies from well to well, the highest being about one barrel per 20,000 cu. ft. and the lowest, one barrel per 1,000,000 cu. ft. of gas. The residual gas is either passed through a scrubbing plant and piped to Calgary, or is permitted to burn in open flares in the valley. Since the production of gas from the wells is many times that of the local requirements, most of the waste gas up to a few months ago was burned in the open flares. The reflection of light at night above the valley could be seen for miles, and advertised far and near the magnitude of the wastage of fuel connected with the recovery of a small fraction of liquid fuel from the major resource of natural gas arising from the wells of the valley. Restrictive measures on the part of a conservation committee of the provincial government are designed to bring about a larger recovery of liquid hydrocarbons from the wet gas, and to conserve the gas resources of the valley.

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The crude naphtha from the separators contains highly volatile hydrocarbons and sulphur compounds which make it desirable to refine the naphtha before using it as a motor fuel. At present the general practice in the valley is to "weather" the crude naphtha in tanks open to the air, or even to heat the tank contents with steam in order to drive off the more volatile hydrocarbons, principally propane and butanes. This involves a loss of up to 30% of liquid hydrocarbons, the average loss in 1930 being probably in the neighborhood of 12% of the liquid naphtha recovered in the separators. No recovery of these valuable hydrocarbons has as yet been attempted, although elsewhere on the continent both butanes and propanes are recovered and sold as fuel or for lighting purposes. The process of removing these volatile hydrocarbons is known as "stabilizing" the naphtha.

After "stabilizing", the naphtha is sent to the refinery where it is either mixed with crude oil from other sources and refined, or is treated for the removing or changing of the objectionable sulphur compounds. It is then marketed. The latter treatment is the practice in the smaller refineries, and recently processes have been adopted which provide a fairly satisfactory motor fuel without admixture of gasolines derived from any other source than the Turner Valley naphthas. A certain amount of the naphtha is disposed of as a motor fuel without being refined. If such a fuel comes directly from the separator the volatile hydrocarbons are largely lost before they enter the combustion chamber of the engine. This objection cannot be raised with regard to the naphtha from the stabilizers but, whether stabilized or not, crude naphthas contain the objectionable sulphur compounds. Whether these sulphur compounds give rise to corrosion or not is a matter of opinion rather than of demonstration, but the odor of hydrogen sulphide and mercaptans makes them undesirable constituents of automotive fuels.

Several analyses of crude naphtha from the wells of Turner Valley have been made by Mr. R. Rosewarne, engineer of the Fuels and Fuel Testing Division of the Department of Mines, Ottawa. These show a propane content varying from 2 to 10.8%; butanes, 8.8 to 20.3%; pentanes and higher hydrocarbons, 61.4 to 89.2%. Methane and ethane, up to 5% of each, are also constituents of some of the crude naphthas. The specific gravity ranged from 0.62 to 0.70. No data respecting the sulphur compounds, excepting the total sulphur content of the crude naphthas, were obtainable. The total sulphur content of the crude naphthas was, however, known to range between 0.10 and 0.20%.

Corrosion of telephone lines and telephone apparatus adjacent to the Turner Valley field has been reported from time to time, the corrosion being attributable to the sulphur compounds emitted into the air from leaking gas pipes, blowing of wells, the gases from stabilizer tanks and the oxidation products from the open flares in the valley. Corrosion of exposed metal parts in the operating plants in the valley was also observed but, considering the exposure to which these were subjected, the damage was relatively small. This relative freedom from corrosion is probably due to the lack of

moisture in the air and not to the absence of sulphur compounds. Tanks and pipes covered with aluminium paint appeared to be well protected against corrosive attack. No evidence could be obtained from the condition of the vegetation in Turner Valley that there was a sufficient concentration of sulphur dioxide in the atmosphere to adversely affect the growth of plants. At all times and places the vegetation appeared normal.

The investigation described in this paper was carried out for the purpose of learning something of the nature of the sulphur content of the Turner Valley naphthas, particularly in relation to refinery practice. The refining processes are largely concerned with either removing the sulphur compounds from the naphtha, or rendering them non-objectionable from the standpoint of odor and corrosion, and in securing a product free from color and the tendency to gum formation in storage.

Material

Through the courtesy of Mr. W. Calder, Chief of the Petroleum and Natural Gas Division of the Provincial Department of Lands and Mines, and Mr. C. W. Dingman, District Petroleum and Gas Engineer of the same department, samples were secured from the separators attached to four representative wells, *viz.*, McLeod No. 4, Sterling Pacific No. 1, Spooner No. 1 and East Crest Nos. 1 and 2A combined. The samples were taken direct from the bottom of the gauge glass of the separators and kept in brown glass bottles previously well scrubbed with the gas from the separators, and at no time exposed to the air or to the light. Small portions for analysis were removed from the containers by making use of the pressure generated by warming the bottles slightly.

Experimental

Since the investigation was not concerned with distilling practice the separate samples were not examined for volatility, but a 100-cc. sample made up of equal parts of the four samples was distilled according to standard practice. The first drop appeared at 30° C.; 10% came over between 30° and 50°; 15% between 50° and 55°; 10% between 55° and 70°; 10% between 70° and 80°; 10% between 80° and 90°; 10% between 90° and 100°; 20% between 100° and 120°; and 10% between 120° and 165° C. The liquid residue (5%) was yellowish in color but no solid separated on cooling. The distillates were colorless but on standing for several weeks exposed to the light a slight yellow color developed in the last fraction. The specific gravity of the mixed naphthas before distilling was 0.687 at 20° C.

Sulphur Content

All four samples reacted strongly with doctor solution (sodium plumbite) forming, without the addition of sulphur, yellow to black precipitates, and the naphthas on filtering were yellow. On the addition of sulphur all precipitates became brown or black, excepting the East Crest sample which

changed from yellow to brown. The presence of hydrogen sulphide and disulphides in all of the samples modified the characteristic color reactions of lead plumbite on the mercaptans.

All four naphthas contained alkyl disulphides and mercaptans and all gave positive reactions to the indophenene test, indicating the presence of thiophenes.

An approximate analysis to determine the various kinds of sulphur compounds present in the naphthas was carried out in accordance with a method suggested by Youtz and Perkins (7). The total sulphur was determined by the standard combustion method using a burner and absorption pipette. The sample (25 cc.) was then shaken for 20 min. with an equal volume of a saturated aqueous solution of sodium carbonate to remove the hydrogen sulphide, and the sulphur again determined in the naphtha by combustion. The disulphides were then removed by shaking the naphtha for 20 min. with an equal volume of a saturated aqueous solution of sodium sulphide, and the residual sulphur determined in the naphtha by combustion. Finally, the naphtha was shaken with an equal volume of a saturated aqueous solution of mercuric chloride to remove mercaptans, the residual sulphur in the naphtha again being determined as above. After each treatment the naphtha was washed twice with water before the sulphur was determined. The results according to this approximate separation are shown in Table I.

TABLE I
AMOUNTS OF VARIOUS KINDS OF SULPHUR COMPOUNDS PRESENT IN
TURNER VALLEY NAPHTHAS

Sample	% Total S	% S as H ₂ S	% S as di-sulphides	% S as mercaptans	% S undetermined
McLeod No. 4	0.162	0.057	0.044	0.032	0.029
Sterling Pacific No. 1	0.162	0.027	0.031	0.080	0.024
Spooner No. 1	0.148	0.050	0.053	0.022	0.023
East Crest Nos. 1 and 2A	0.186	0.055	0.041	0.068	0.022

After the removal of the sulphides and disulphides, all the naphthas gave the characteristic canary yellow coloration with sodium plumbite; they became orange-colored and yielded a precipitate on the addition of sulphur. This proved conclusively the presence of mercaptans. After shaking with mercuric chloride solution, no positive test for either mercaptans or thiophenes could be obtained. The thiophenes therefore are contained in the mercaptan sulphur, but all tests for thiophenes indicated their presence in but very small quantities.

Corrosion tests on the untreated naphthas, using the copper strip method, were all positive and the order of intensity in the blackening of the copper was, first, Sterling Pacific, followed by Spooner, McLeod and East Crest in the order named. Shaking with mercury produced a heavy black precipitate.

Treatment with Sulphuric Acid

Samples of the four gasolines were placed in wash bottles and treated with an equal volume of 96% sulphuric acid. Air was drawn through in such a manner as to mix the gasoline and acid. After 30 min. the samples were washed with a saturated aqueous solution of sodium carbonate and with water, and analyzed for sulphur. The sulphur content in the McLeod No. 4 and Spooner No. 1 samples was reduced to about 0.01% by this treatment, while in Sterling Pacific and East Crest it was reduced to 0.04%. All the treated naphthas were negative to doctor solution. The air drawn through the mixture of acid and naphtha was passed into a standard alkali solution and the volume of sulphur dioxide evolved determined. The amount of sulphur dioxide evolved was negligible. Apparently this treatment was very effective for the removal of sulphur when it was not combined as mercaptans. Both Sterling Pacific and East Crest were relatively high in mercaptans.

Samples of the four naphthas were mixed in equal proportions and a series of experiments carried out to find the minimum quantity of sulphuric acid required, with 20 min. shaking, to remove the sulphur compounds. A 25-cc. sample of the mixed naphthas was first shaken with an equal volume of 20% sodium hydroxide solution and washed with water. The minimum proportions by volume were found to be about 1 of 96% acid to 15 of the naphtha. A smaller proportion of concentrated acid, or dilute acid, required a longer period of contact before the naphtha was entirely sweetened. The sulphur content of the mixed naphthas after treatment was about 0.01%.

During these experiments it was found that only a very small loss, 2% by volume, of naphtha occurred if the temperature of the mixture was kept below 20° C. The reaction with sulphuric acid produced considerable heat and the sulphuric acid was blackened, but very little evolution of sulphur dioxide took place.

The naphthas sweetened by the sulphuric acid treatment and washed with sodium hydroxide solution and water were kept under observation for over a year. The water-white products gradually assumed a brownish yellow color and there was a small deposit of gum. The samples were exposed on the laboratory table. Acidity to methyl orange developed but the samples remained sweet to doctor solution. They developed however a highly corrosive action towards copper. This action could be prevented by washing with sodium hydroxide solution. No blue color was formed on shaking with potassium iodide-starch solution.

Treatment with Sodium Hypochlorite

A sodium hypochlorite solution was prepared by saturating in the cold a 20% solution of sodium hydroxide with chlorine and then making it alkaline by the addition of one-fifth of its volume of the 20% alkali solution. Neither the alkalinity nor the available chlorine was determined. Equal volumes (25 cc.) of the freshly prepared sodium hypochlorite solution and of the naphthas were shaken together in 100-cc. flasks for 20 min., separated, and then the naphthas were washed twice with water. Three of the naphthas

were not rendered completely sweet by the above treatment but longer contact with the sodium hypochlorite solution reduced the sulphur content and the positive test towards doctor solution. The single 20-min. treatment rendered Spooner No. 1 naphtha sweet and reduced the sulphur content to 0.05%, while in the other three samples it was reduced to about 0.10%. The sodium hypochlorite treatment preceded by a caustic wash with sodium hydroxide solution rendered all four of the naphthas sweet and reduced the sulphur content to about 0.05%. Possibly a more strongly alkaline solution of sodium hypochlorite or longer contact with the sodium hypochlorite solution as used would have rendered the naphthas sweet without a previous caustic wash.

The naphthas were rendered water-white by the sodium hypochlorite treatment but developed a yellow color on standing for a few hours. This color developed much more slowly when the samples were kept in the dark.

Naphthas treated with sodium hypochlorite, unless followed by a caustic wash, were found to give a heavy black precipitate with mercury. Treatment with chlorine gas was attempted but the experiments were not continued as there was a tendency for the naphthas to emulsify when washed with alkali or water.

Treatment with Cupric Chloride

Samples of the naphthas were given a preliminary wash with sodium hydroxide solution, followed by shaking for 20 min. with an equal volume of a saturated aqueous solution of cupric chloride. The treated naphthas, after washing with water, were sweet to doctor solution but the sulphur content was very little reduced. There was a tendency towards emulsifying on shaking with the cupric chloride solution. A previous wash with sodium hydroxide solution prevented the blackening of the cupric chloride solution due to the removal of the sulphides. After treatment the naphthas were filtered through "diotite", a diatomaceous earth, and set aside for observation. They slowly became yellow and gum formation was observed, but they remained sweet towards doctor solution for over a year.

Peroxides

Qualitative tests were made for peroxides, using titanous chloride, but no reaction could be detected. Samples of naphtha left exposed to light for six months in stoppered bottles containing air also failed to show any formation of peroxides. The four naphthas were also tested by shaking with potassium iodide solution to which a little starch was added. None gave a blue coloration. Apparently the naphthas do not contain any strongly oxidizing compounds.

Coloration

The samples of naphtha when collected, and after keeping for over a year in brown glass bottles, had very little color. McLeod No. 4 and Sterling Pacific had a slight yellowish tinge, while Spooner No. 1 was just faintly yellow, and East Crest was water-white. Samples of these naphthas were set aside for observation of the color changes under varying conditions.

Set No. 1 was kept in stoppered glass bottles, but no attempt was made to keep them air-free. They were exposed on the laboratory table at times to direct sunlight and strongly lighted at all times during the day. A yellow color developed in a few days, the depths of color being in the order mentioned above. After a few months, gum was observed to form on the sides and bottom of the flasks, and the amount appeared to be in the order of the depth of color. East Crest after a year's exposure was only faintly yellow and gum formation was not marked.

A second set of samples was placed in a dark cupboard. These developed color very slowly and no gum formation could be observed after a year's exposure.

A third set was wrapped in black paper and set aside in the cupboard. No color development or gum formation whatsoever could be observed.

A fourth set was exposed to the sunlight for a day and then placed in the dark. No further development of color occurred.

Samples of naphtha which were placed in bottles in which the air had been displaced by carbon dioxide became yellow and gum was formed, as was the case when the samples were in contact with air. Evidently the color development and gum formation is attributable to exposure to the light and not to the presence of air.

A mixed sample of four naphthas was distilled and various fractions set aside. The fractions distilling below 165°C . were colorless and remained so for eight months when exposed to the same light conditions as was set No. 1. No gum formation occurred in these fractions. Fractions above 165°C . were yellowish, the color deepening to brown as the distillation proceeded. The color of these fractions deepened under the light conditions mentioned above, gum formation occurred and a black deposit formed in the higher fractions.

Filtering the colored naphthas through "diotite" removed the yellow color, but on exposure to light the color again developed and gum formation took place.

Corrosion

The four naphthas were strongly corrosive to copper and gave heavy black deposits when shaken with mercury. A caustic wash followed by water removed the corrosive compounds or changed them so that the copper test was negative. This accounts for the fact that several of the refineries now treat Turner Valley naphthas with an alkali wash, and market the product without any other treatment. The sulphuric acid treatment followed by a caustic wash gave a naphtha at first non-corrosive to copper, but on standing for some time it became corrosive. Treatment of the naphtha with sodium plumbite, without the addition of sulphur, leaves the naphthas with a canary yellow color, and elemental sulphur must be added to change these yellow compounds to colorless. A slight excess of free sulphur over the amount necessary makes the naphtha decidedly corrosive. Consequently the doctor treatment must be very carefully regulated in regard to the addition of sulphur.

Treatment with free chlorine or with sodium hypochlorite and washing with alkali solution gave a non-corrosive product, providing the washing was thorough. It was found however that, on standing, a yellow color developed in the naphtha and the corrosion test became positive.

Treatment of the naphthas with sodium hydroxide solution followed by a saturated cupric chloride solution and washing gave a sweet non-corrosive product, remaining so for 12 months.

Filtering through "diotite" did not remove the corrosive substances in the naphthas nor were they rendered sweet to sodium plumbite.

Discussion

The four naphthas examined had a total sulphur content ranging from 0.148% to 0.186%, and consisting of varying amounts of sulphides, disulphides and mercaptans. A caustic soda wash removed the sulphides and probably some of the lower mercaptans, reducing the sulphur content to the neighborhood of 0.1% and giving a non-corrosive product which remained colorless for a time. A large part of the mercaptan content, however, remains in the naphtha as such and, on standing, a yellow color develops and gum formation occurs. Nevertheless this refining process, becoming more popular in the treating of Turner Valley naphthas, gives a gasoline much superior to the crude casinghead naphtha or the "stabilized" naphtha marketed in the valley. The practice of coloring the naphtha blue masks the yellow color that develops on standing. It is extremely interesting that the caustic wash should render the gasoline negative to the copper strip corrosion test, when only half or less than half of the total sulphur content has been removed. The advantage of the caustic wash over other methods lies in its simplicity and cheapness, making it possible to produce a salable product at a considerably lower price than if the naphtha were mixed with crude oil or gasolines from other sources and refined by more expensive processes. The disadvantage of alkali-washed Turner Valley naphthas lies in the fact that they still contain some of the objectionable odorous sulphur compounds (mercaptans) and too high a content of volatile hydrocarbons.

Turner Valley naphthas are susceptible to the sodium hypochlorite treatment following a caustic wash, which transforms the mercaptans to the less objectionable disulphides, removes the sulphides and reduces the sulphur content to 0.05% (3, 4, 5, 6). Naphthas so treated remained sweet but developed a yellow color in time, and gum formation followed. The cupric chloride treatment following a caustic wash rendered the naphthas sweet and the development of a yellow color and gum formation seemed to be retarded, but the sulphur content was not reduced to as low a proportion as with the sodium hypochlorite treatment. Nevertheless the naphthas treated with cupric chloride remained non-corrosive for over a year.

The alkaline plumbite (doctor solution) treatment accompanied by the addition of sulphur renders the naphthas sweet, but care must be exercised in the addition of the sulphur to avoid any excess, as even a slight excess was found to render the naphthas corrosive.

Treatment with sulphuric acid followed by a caustic wash produces water-white naphthas, sweet for an indefinite period and having a very low sulphur content. On standing, however, a yellow color slowly develops, accompanied by gum formation. Previous washing with sodium hydroxide solution would diminish the amount of sulphuric acid required to oxidize the sulphur compounds, but it would be necessary to find out whether such additional treatment would be cheaper in practice. No excessive loss of hydrocarbons follows the use of concentrated sulphuric acid, providing the temperature is kept down to 20° C.

None of the treatments carried out in this investigation produced a naphtha remaining colorless for an indefinite time (1, 4). Neither did any of the treatments render the naphtha free from the development of the objectionable gummy compounds. Exposure to light always resulted in the development of a yellow color and the precipitation of gum. On the other hand all crude and treated samples were free from color development and gum formation when protected from the light. It would seem desirable, therefore, whatever the method adopted in refining Turner Valley naphthas, that care should be exercised in protecting the refined product from exposure to light. The development of the yellow color (4) in itself is probably harmless but gum formation is certainly undesirable.

A sulphur content of 0.04% in a gasoline is non-corrosive in motor engines (2), while a content of 0.15% is appreciably corrosive. The Turner Valley naphthas, if untreated, are to be considered as corrosive, while, if subjected to a caustic wash reducing the sulphur content to 0.1%, they are in the threshold region of corrosion where the limits of tolerance have not as yet been established. No doubt refining practice which reduces the sulphur content to 0.05% gives a more desirable product so far as our present knowledge of the relation of sulphur content to corrosion goes, but it is a moot question whether the extra cost of refining required to reduce the sulphur content from 0.1% to 0.05% without removing the tendency to gum formation is justified. Refinery practice which renders the Turner Valley naphthas relatively odorless and reduces the sulphur content to about 0.1% ought to be satisfactory, providing the refined product is not later exposed to light. Coloring the naphtha will mask the yellow color developed on exposure to light but will not prevent the formation of gum. Distillation concentrates the color and gum producing properties in the high boiling fractions. If the separation of the propane and butanes was economically practicable, refining practice for Turner Valley naphthas would probably be confined to a caustic wash with or without sodium sulphide or polysulphides followed by distillation. The first distillates would contain the propane and butanes, from 5 to 30%; the middle fraction up to 165° C. from 65 to 90%, marketable gasoline, and a high boiling fraction of about 5% containing the colored and gum producing properties could be treated along with the crude oils produced in the valley. A comparison of methods for treating the crude oils of the valley has not as yet been made.

It is recognized that other factors, particularly economic ones, enter into the choice of a method of refining Turner Valley naphthas. It is also recognized that this investigation falls far short of determining the proper concentrations of reactants or the optimum conditions under which these reactants would be most effective in refining practice, but the results at least indicate what treatments are most likely to give to the consumer a refined gasoline which is satisfactory from the standpoints of sulphur content, corrosion, color and gum formation.

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STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES

XLIV. SYNTHESIS OF ISOMERIC BICYCLIC ACETAL ETHERS¹

By EARLAND G. HALLONQUIST² AND HAROLD HIBBERT³

Abstract

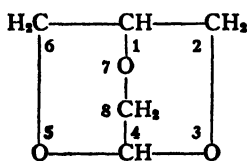
Two isomeric bicyclic acetal ethers, namely, 3, 5, 7-trioxabicyclo [2, 2, 2] octane* and 3, 6, 8-trioxabicyclo [3, 2, 1] octane, have been synthesized by the action of aqueous potassium hydroxide on 1, 3-bromoethylidene glycerol 2-benzoate and 1, 2-bromoethylidene glycerol 3-benzoate respectively. Both isomers are crystalline products possessing similar solubilities in organic solvents and high vapor pressures. They show no indication of interconvertibility on solution in solvents, or under the influence of heat. On hydrolysis they yield glycollic aldehyde and glycerol.

A new method for the synthesis of cyclic acetals from simple open-chain acetals is described.**

Introduction

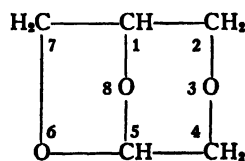
For a thorough understanding of the nature and properties of carbohydrates, and especially of polysaccharides, three factors call for especial consideration, namely: (1) the position in space of the hydroxyl groups; (2) the nature of the carbon-oxygen valence angle in the ring and its bearing on the stability of the latter; (3) the nature and tendency towards polymerization of carbon-oxygen ring compounds, and the stability and polar character of the resulting polymerized product.

Some ten years ago (8) preliminary experiments were carried out on the action of alkalis on bromoethylidene glycerol and a crystalline product isolated to which was tentatively assigned the structure of (A) or (B).



(A)

3, 5, 7-Trioxabicyclo [2, 2, 2] octane



(B)

3, 6, 8-Trioxabicyclo [3, 2, 1] octane

In the light of recent developments on the magnitude of the carbon-oxygen valence angle (1, 6) it seemed of interest again to take up the study of this subject with the object of securing additional information on the structure and properties of -C-O-C- ring linkages, and to investigate first of all bicyclic derivatives containing neither hydroxyl nor carbonyl groups.

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* This system of nomenclature is in accordance with that suggested by the Division of Chemical Abstracts, American Chemical Society.

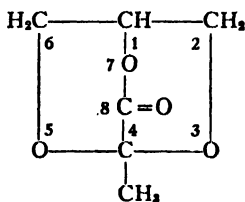
** This method is to be applied later to carbohydrates and related compounds.

It has been found that the action of aqueous potash on 1, 3-bromoethylidene glycerol 2-benzoate and 1, 2-bromoethylidene glycerol 3-benzoate yields two isomeric substances, namely, (A) and (B) respectively, the structure of which has been definitely settled by the procedure outlined in the Experimental Part.

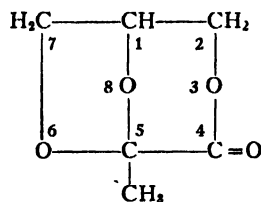
The bicyclic derivative (A) is formed much more readily than (B) and this property can, in fact, be utilized for the isolation of 1, 2-bromoethylidene glycerol from a mixture of the bromoethylidene derivatives. Both isomers have a well-marked crystalline character, a high vapor pressure, and similar solubilities in organic solvents, while neither shows any tendency to pass over into the other in solution or under the influence of heat.

It is of interest that a structural model of (A) employing the same valence angle for $-C-O-C-$ as for $-C-C-C-$ linkages represents a highly symmetrical substance.

The fact that interconversion of the one into the other does not readily take place is probably to be associated with the absence of strongly polar groups, inasmuch as Haskellberg (4) has recently found that on heating together glycerol and pyruvic acid two isomeric bicyclic derivatives, namely, 4-methyl-3, 5, 7-trioxabicyclo [2, 2, 2] octan-8-one (C) and 5-methyl-3, 6, 8-trioxabicyclo [3, 2, 1] octan-4-one (D), are formed, one of which is a solid (C) and the other a liquid (D) and to which the author has arbitrarily assigned the following structures:



(C)

4-Methyl-3, 5, 7-trioxabicyclo
[2, 2, 2] octan-8-one

(D)

5-Methyl-3, 6, 8-trioxabicyclo
[3, 2, 1] octan-4-one

without, however, providing any experimental proof. The point of interest is the discovery that the liquid isomer (D) on being allowed to stand in the presence of hot water passes over into the isomer (C), which separates out in the crystalline state on cooling.

A study of this new type of compound is of interest from the point of view of the stability of heterocyclic carbon-oxygen ring compounds, the carbon-oxygen valence angle in heterocyclic rings, the properties of polysaccharides, and the Hibbert-Michael ring partition principle.

Synthesis of Cyclic from Simple Open-chain Acetals

Due to the difficulty in preparing various substituted halogen and hydroxy-cyclic acetals, it was found desirable to devise a new method for their synthesis. The process described consists in merely heating a mixture of the polyhydroxy

derivative (for example, glycerol) with an open-chain acetal (for example, chloroacetal), upon which the lower-boiling alcohol distills over leaving the desired cyclic acetal in good yield.

Using this method the following products have been prepared: chloroethylidene glycerol, -glycol, -trimethylene glycol; β -chloropropylidene glycerol and β -bromopropylidene glycerol.

Experimental

Preparation of 3, 5, 7-Trioxabicyclo [2, 2, 2] octane (A)

1, 3-Bromoethylidene glycerol 2-benzoate, the preparation of which is given in detail in a previous communication by the authors (7), m.p., 109° C., (40 gm.), was mixed with a solution of 4.5 gm. of potassium hydroxide in 100 cc. of water in a three-necked flask fitted with mercury seal stirrer and reflux condenser. The contents was stirred vigorously at 100° C. and 50% potassium hydroxide solution added at intervals as follows: after 2 hr. heating, 6 gm.; after 2½ hr. heating, 7.0 gm.; after 2¾ hr. heating, 6.0 gm.; after 4 hr. heating, 6.0 gm.; after 5 hr. heating, 6.0 gm.

Solution of the benzoate took place after about an hour's heating and stirring. After all the potassium hydroxide had been added, the solution was heated for 30 min., then cooled and saturated with potassium carbonate. The precipitated potassium benzoate was filtered off, and both precipitate and filtrate extracted six times with ether. The ether extract was dried, and the ether removed, the residue solidifying completely. M.p., 99° C. Yield, 14.8 gm. (95%).

This solid gave a slight test for halogen. It was purified by heating with 25% potassium hydroxide under reflux at 100° C. for ten hours. Saturation with potassium carbonate and extraction with ether yielded a crystalline solid free from halogen. It recrystallized from warm ligroin (b.p., 60-70° C.) in ice-flower formation; m.p., 99° C. The solid possesses a high vapor pressure, a small sample placed on a watch glass disappearing completely in a few hours' time. It is very soluble in water, alcohol, ether, ethyl acetate, chloroform, toluene, benzene, glycol monomethyl ether and dioxane, less soluble in ligroin and carbon tetrachloride. Analysis:—Found: H, 6.8, 6.8; C, 51.6, 51.7%. Calcd. for $C_8H_8O_3$: H, 6.93; C, 51.68%. Mol. wt. (ethylene bromide). Found: 117, 115. Calcd. for $C_8H_8O_3$, 116.

The product gave no test indicating unsaturation and when treated with benzoyl chloride in pyridine solution gave no benzoate, the original substance being recovered unchanged.

Examination for Hydroxyl Groups with Magnesium Methyl Iodide Solution

Application of the method of Hibbert (5) indicated absence of any hydroxyl groups in the product. A weighed sample was dissolved in isoamyl ether and treated with an excess of methyl magnesium iodide in the same solvent, in a closed apparatus, in which the volume of any methane evolved could

be carefully measured; 0.2004 gm. (assuming one hydroxyl group per mole) should give 38.7 cc. of methane at N.T.P. Found, 1.7 cc., presumably due to a trace of moisture or impurity.

Hydrolysis of 3, 5, 7-Trioxabicyclo [2, 2, 2] octane (A)

This compound (5.056 gm.) was mixed with 20 cc. of 0.01 *N* sulphuric acid and the solution heated at 70° C. for ten hours. It was then cooled, almost neutralized with very dilute sodium hydroxide, and made up to a volume of 50 cc. (E). A small sample treated with ammoniacal silver nitrate gave a silver mirror immediately. The quantity of aldehyde group present was determined by the iodine oxidation method of Willstätter and Schudel (11). Analysis showed 95% of the theoretical quantity of aldehyde present.

Identification of Glycollic Aldehyde

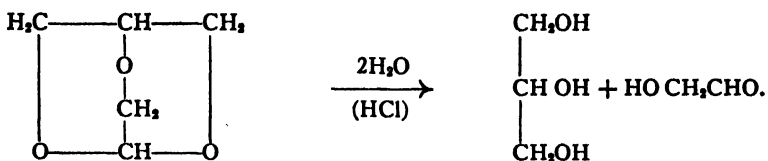
A sample of the original hydrolysis liquor (E) was added to a saturated alcoholic solution of *p*-nitrophenylhydrazine and the solution heated at the boiling point for a minute. A drop of concentrated hydrochloric acid was then added and the solution heated for a minute longer. It rapidly turned from orange to red, and a brick-red flocculent precipitate formed. The mixture was filtered, and the precipitate washed thoroughly with 50% alcohol and dried. It was insoluble in water, alcohol, ether, ligroin, ethyl acetate and chloroform, but soluble in pyridine, aniline, nitrobenzene, etc. It crystallized from pyridine in wine-red needles. These were filtered and washed thoroughly with ether. The dried, brick-red colored product gave a deep blue solution with alcoholic alkali. It softened at 290° C. and melted at 309-311° C. with evolution of gas, and was identical in properties with the *p*-nitrophenylosazone of glycollic aldehyde obtained by Wohl and Neuberg (12) and others (2, pp. 214-228; 9).

Isolation of Glycerol from the Hydrolysis Liquor (E)

The hydrolysis liquor (E, 40 cc.) was treated with 100 gm. of 5% sodium amalgam and the required amount of dilute sulphuric acid, the latter being added gradually over a period of two hours. The clear solution was poured off from the mercury, carefully neutralized, and this evaporated as completely as possible under reduced pressure (30° C. and 20 mm.). The residue was extracted with absolute methyl alcohol, the latter removed and the syrupy residue distilled (2 mm.). Most of the distillate (2 gm.) came over at 143-147° C., a small amount of charred product remaining in the flask. The fraction (b.p., 143-147° C./2 mm.) was then converted into the phenylurethane by heating one gram with four grams of phenyl isocyanate. The reaction mixture was heated on the steam bath for two hours, when crystallization took place and the whole mass became solid. This product was washed with ligroin to remove excess phenyl isocyanate and then dissolved in hot alcohol. On cooling and standing a white amorphous solid separated which was filtered off and dried (m.p., 181° C.). It could not be crystallized from any of the common solvents. The triphenylurethane of glycerol prepared

similarly was an amorphous, white solid, melting at 180° C. (from ethyl alcohol). A mixture of this with the previous product melted at 181° C.

The products of hydrolysis of 3, 5, 7-trioxabicyclo [2, 2, 2] octane are thus glycollic aldehyde and glycerol, hydrolysis at the acetal and ether linkages taking place simultaneously.



3, 5, 7-Trioxabicyclo [2, 2, 2] octane

Preparation of 3, 6, 8-Trioxabicyclo [3, 2, 1] octane (B)

(a) Preparation of Glycerol α -Benzoate

Glycerol α -benzoate was prepared according to the method of Fischer and Bergmann (3) with certain improvements resulting in a higher yield. Isopropylidene glycerol benzoate (100 gm.) (prepared by benzoylating isopropylidene glycerol) was mixed with 450 cc. of 0.075 *N* hydrochloric acid and stirred vigorously with an automatic stirrer at 55-60° C. until solution took place and the turbidity just disappeared. This required about two hours. The solution was immediately cooled, neutralized with potassium bicarbonate, saturated with sodium chloride and extracted repeatedly with ether. The ether extract was dried over anhydrous sodium sulphate. On distilling off the ether, the glycerol α -benzoate remained behind as an oil. After drying for some time in a vacuum desiccator over phosphorus pentoxide, a small sample was cooled with a solid carbon dioxide ether mixture when crystallization took place. The main portion of the oil was seeded with these crystals and rapidly solidified. The solid glycerol α -benzoate was recrystallized from a solvent consisting of 99% of anhydrous ether and 1% of petroleum ether; m.p., 36° C. Yield, 81.5 gm. (98%). The highest yield obtained by Fischer and Bergmann was 63%.

(b) Preparation of 1, 2-Bromoethylidene Glycerol 3-Benzoate

Crystalline tribromoparaldehyde was prepared in 15% yield by the bromination of paraldehyde, following, in general, the procedure of Stepanow (10) but using a few drops of concentrated sulphuric acid as a catalyst, and allowing the reaction mixture to stand at 0° C. for several days. The tribromoparaldehyde, after recrystallization from alcohol and ligroin (b.p., 80-90° C.) melted at 103-104° C.

To 101 gm. of pure crystalline tribromoparaldehyde was added 182 gm. of pure crystalline glycerol mono α -benzoate and 12 drops of 40% sulphuric acid, and the mixture heated at 90-110° C. for 24 hr. It became homogeneous and liquid upon short heating, and at intervals of about four hours a vacuum was applied to the flask to remove water formed in the reaction. The reaction mixture was cooled, washed several times with water, extracted with ether,

the ether extract washed twice with dilute sodium hydroxide solution, once with concentrated sodium bisulphite solution, three times with water, and then dried over anhydrous sodium sulphate. After removal of solvent, distillation of the residual liquid yielded 130 gm. (52%) of 1, 2-bromoethylidene glycerol 3-benzoate; b.p., 169-171° C./1 mm. n_D^{20} , 1.5449.

(c) *Preparation of 3, 6, 8-Trioxabicyclo [3, 2, 1] octane (B)*

Experiment 1. Pure 1, 2-bromoethylidene glycerol 3-benzoate (50 gm.) was mixed with a solution of 5 gm. of potassium hydroxide in 200 cc. of water in a three-necked flask fitted with a mercury seal stirrer and reflux condenser and the product stirred vigorously at 100° C. for two hours. Further quantities of 3 gm. of potash in 5 cc. of water were then added after intervals of 2, 4, 6 and 7 hr. respectively. After a total of 15 hr. of stirring at 100° C., the contents of the flask was cooled, saturated with potassium carbonate and extracted repeatedly with ether. The ether extract was dried over anhydrous sodium sulphate, the ether removed and the residue fractionated. Two products were obtained:—

(a) A substance distilling at 68-70° C./12 mm. and which solidified on cooling; m.p., 56-57° C. Yield, 2 gm. (10%).

(b) A substance distilling at 141-142° C./12 mm. n_D^{25} , 1.5006. Its properties corresponded with those of 1, 2-bromoethylidene glycerol as found in a previous investigation (7). Yield, 24 gm. (75%).

The recovery of such a large amount of the acetal indicates its comparative inertness towards alkali as compared with the isomeric 1, 3-bromoethylidene glycerol.

Experiment 2. In order to ascertain whether the yield of bicyclic product could be increased, a second experiment was performed with more concentrated alkali using 75 gm. of the 1, 2-bromoethylidene glycerol 3-benzoate and a solution of 13 gm. of potassium hydroxide in 150 cc. of water. The mixture was stirred vigorously at 100° C. for one hour and two lots of 13.5 gm. each of potash dissolved in 15 cc. of water added after one hour, and three hours' heating respectively, followed by a third and fourth addition of 10 gm. of potash dissolved in 10 cc. of water after 4½ and 5½ hr. respectively.

At the end of six hours the oily benzoate had completely dissolved. Heating at 100° C. was then continued for an additional 13 hr. The solution was cooled, saturated with potassium carbonate, the precipitated potassium benzoate filtered off, and both precipitate and filtrate extracted repeatedly with ether. The ether extract was dried and the ether removed. The solid residue left was then distilled very rapidly directly into a flask cooled by running water. Under these conditions the entire residue distilled at 68-70° C./12 mm. yielding 9 gm. of a colorless crystalline solid; m.p., 56-57° C. Yield, 31%. No 1, 2-bromoethylidene glycerol was obtained as in the previous experiment. About 60 or 70% of the reaction products were ether insoluble

and remained in the alkali liquor, unextracted by the ether. Extraction with alcohol was impracticable due to the presence of large quantities of potassium benzoate.

Experiment 3. A third experiment was conducted under the same conditions as above, but using 1, 2-bromoethylidene glycerol (*i.e.*, the hydrolyzed product) instead of 1, 2-bromoethylidene glycerol 3-benzoate. The reaction product was neutralized with 10% sulphuric acid, and evaporated to dryness under reduced pressure. The bicyclic product formed in the reaction was lost at this point due to its high volatility in presence of water vapor. The residue was extracted with alcohol. After removal of the latter a viscous syrup was left which contained no halogen. On distillation under reduced pressure a portion distilled over a wide range (135-175° C./3 mm.) with some decomposition; the residue, a semisolid resinous mass, could not be distilled. This was not investigated further.

The white crystalline solid (m.p., 56-57° C.) obtained from the ether extracts in experiments Nos. 1 and 2 was entirely free from halogen. It showed a similar behavior towards organic solvents as the previous isomer (A). It was saturated, contained no hydroxyl groups and was readily volatile, small quantities left exposed to the open air disappearing in a short time (one or two hours). Analysis:—Found: H, 6.8, 7.0; C, 51.5, 51.7%. Calcd. for $C_8H_8O_3$: H, 6.93; C, 51.68%. Mol. wt. (ethylene bromide):—Found: 118, 120. Calcd. for $C_8H_8O_3$, 116.

Hydrolysis of 3, 6, 8-Trioxabicyclo [3, 2, 1] octane (B)

This product (m.p., 56-57° C.) was hydrolyzed exactly as in the case of the corresponding isomer (A). Glycollic aldehyde and glycerol were identified as the products of hydrolysis.

The action of aqueous alkalis on the isomeric β -chloropropylidene glycerols as well as on the corresponding α , β -dibromo compounds is being investigated. The latter investigation will be of interest as indicating a new application of the Hibbert-Michael ring partition principle.

NEW METHOD FOR THE SYNTHESIS OF CYCLIC ACETALS

Preparation of Chloroethylidene Glycerol

Anhydrous glycerol (30 gm.) and 50 gm. of chloroacetal were heated together with stirring at 115° C. In 10 or 15 min. the mixture had become homogeneous, and a short fractionating column and condenser were then attached to the flask and the temperature gradually raised to 160° C. Ethyl alcohol distilled over (b.p., 78° C.). Yield, 26.5 gm. (88% of theory). The residual oil was distilled under reduced pressure. Yield of chloroethylidene glycerol, 40 gm. (80%); b.p., 130-134° C./13 mm. Analysis:—Calcd. for $C_8H_9O_2Cl$; Cl, 23.02%. Found: 23.21%. In a similar manner chloroethylidene glycol (b.p., 57° C./13 mm.), chloroethylidene trimethylene glycol

(b.p., 60-62° C./11 mm.), β -chloropropylidene glycerol (b.p., 129-135° C./15 mm.) and β -bromopropylidene glycerol (b.p., 136-140° C./18 mm.) were prepared in yields of 40, 60, 56 and 65% respectively.

The method is being applied to the synthesis of other cyclic acetals and its use for determining the structure of carbohydrates and polysaccharides is under investigation.

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THE CONDENSATION OF CERTAIN γ -KETONIC ESTERS WITH AROMATIC ALDEHYDES. II¹

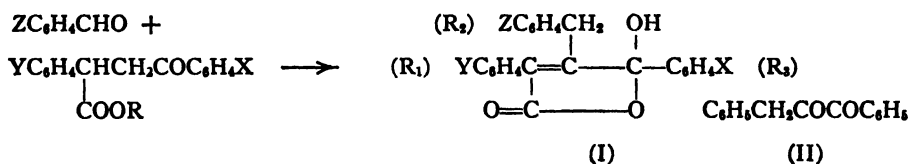
By C. F. H. ALLEN,² G. F. FRAME,³ J. B. NORMINGTON⁴
AND C. V. WILSON⁴

Abstract

The condensation of benzaldehyde with methyl and ethyl α -aryl- β -benzoyl propionates in the presence of sodium methylate, followed by acidification, gave unsaturated ketonic acids. This result was unexpected in view of the previously described similar work in which lactols were formed; the only difference in the molecules is the absence of a substituent group in the benzoyl radical of the ester.

The structure of the acids was carefully determined. On being heated they lost carbon dioxide, forming α , β -unsaturated ketones. One of the latter added hydrogen chloride to give two stereoisomeric saturated chloroketones, which were also synthesized by an independent method.

In an earlier paper (1) several lactols (I) made by alkaline condensation of certain γ -ketonic esters and aromatic aldehydes were described. These lactols were alike in that all had a substituent group in the para position of the third benzene ring (R_3). ($X = \text{Cl, Br, OCH}_3$; $Y, Z = \text{H}$)



When efforts were made to prepare an unsubstituted lactol ($X, Y, Z = \text{H}$) it was found that the product (A) exhibited noticeable differences in chemical properties. In the Grignard machine it showed one active hydrogen and addition of one mole of reagent, while the lactols previously described added two moles of reagent to one active hydrogen.

The substance (A) lost carbon dioxide on being heated, the pyrolysis proceeding in accordance with the equation



This was, indeed, surprising because the lactols did not exhibit this behavior, being unchanged until heated much higher above their melting points and then decomposing completely. At first it was thought that the substance was a β -lactone, although the latter type of compound is not regenerated (as was ours) by acidifying solutions of its salts. Recently (3) it has been shown that in a few instances β -lactones can be formed by acidification of alkali salts, so the fact that our substance was easily recovered on similar treatment did not exclude that type of structure. The pyrolysis product

¹ Manuscript received January 5, 1933.

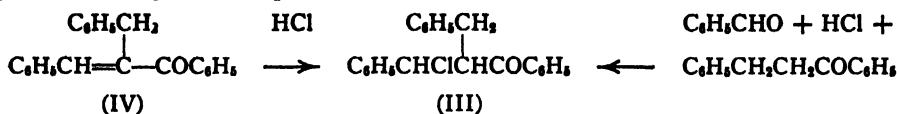
Contribution from the Department of Chemistry, McGill University, Montreal, Canada, with financial assistance from the National Research Council of Canada.

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formed an oily oxime and phenylhydrazone, but gave a crystalline mono-2, 4-dinitrophenylhydrazone, showing the presence of one carbonyl group. On ozonization benzaldehyde was produced. By ozonizing a substance made by pyrolysis of a homologue (B) having a methoxyl group in ring R_1 (Formula I: $Y = OCH_3$, $Z, X = H$) anisaldehyde was obtained, indicating the presence of $R_1CH=$ and showing which R it was. It added hydrogen chloride to form a mixture of the two stereoisomeric chloroketones (III), which were synthesized by an independent method.

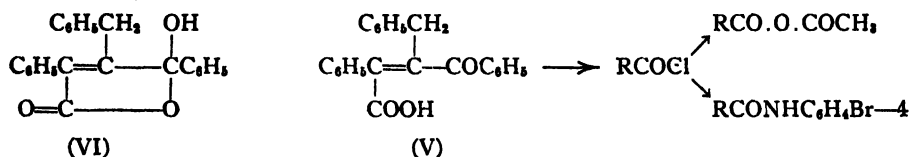


Therefore the pyrolysis product must be α -benzylbenzalacetophenone (IV).

The substance (A) readily formed an oxime and resembled the lactols previously described on oxidation; while permanganate was useless, chromic acid gave an α -diketone, in this case phenyl benzyl diketone (II). This pointed to an essentially similar structure, since when a homologue (B) was prepared with R_1 tagged by a methoxyl group ($Y = CH_3O$) it also gave phenyl benzyl diketone, showing that R_1 was not involved. The formation of this diketone excluded any of the possible β -lactone structures.

It reacted with thionyl but not acetyl chloride; since the product gave a *p*-bromoanilide and a mixed acet-anhydride it must have been an acid chloride.

Collectively these properties point to the structure of an open chain γ -ketonic acid (V) for the substance (A); there was no evidence of the isomeric lactol (VI) as was anticipated in view of the results previously described (1).



It is difficult to find any connection between the presence or absence of a substituent group in the para position of only one ring, and the formation of a cyclic structure in one instance and not in another. It would appear that the substituent must activate the carbonyl group for some, as yet, unexplained reason. Buck and Ide (2) have noted a comparable case of activation of the carbonyl group in *o*-chlorobenzil, the $C=O$ next the chlorine-containing phenyl group being the more reactive—the steric effect was expected to make it less active. The authors are continuing this work using compounds with more varied groups.

Experimental

A. Preparation of the Esters

The methyl and ethyl esters of α -phenyl- β -benzoyl propionic acid were made by refluxing the acid in the appropriate alcohol in the presence of sulphuric acid for four hours. The methyl ester (m.p., 103°C .) was obtained

in a yield of 96%, and the ethyl ester (m.p., 41° C.) in a yield of 71%. The methyl α -(*p*-anisyl)- β -benzoylpropionate was made by the method as outlined in the previous paper (1, ref. 18) from anisalacetophenone; the yields were: nitrile, 93%; acid, 91%; ester, 95% (m.p., 105° C.).

B. Condensation of the Esters with the Aldehydes

This was done by the procedure previously described, except that it was found that the alcohol did not have to be absolute.

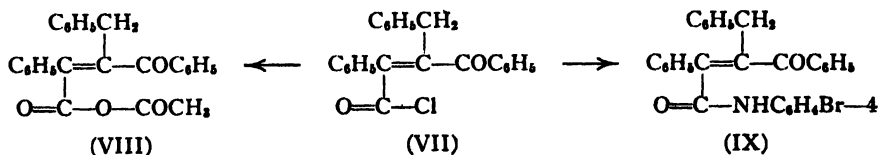
α -Phenyl- β -benzoyl- β -benzyl acrylic acid (V), m.p., 145°C., was very soluble in the usual organic solvents except petroleum ether, and crystallized in plates. The yield was 85% from the methyl ester and 87% from the ethyl ester. It evolved carbon dioxide from warm sodium bicarbonate solution and was precipitated unchanged on addition of acid. The corresponding methoxy homologue, α -(*p*-anisyl)- β -benzoyl- β -benzyl acrylic acid (B) melted at 136° C. The use of anisaldehyde, piperonal and *p*-chlorobenzaldehyde gave thick oily acids. Analyses: Calcd. for (V) $C_{23}H_{18}O_3$: C, 80.7; H, 5.2%; (B) $C_{24}H_{20}O_4$: C, 77.4; H, 5.4%. Found: (V) C, 80.4; H, 5.2%; (B) C, 77.2; H, 5.3%. In the Grignard machine it reacted with two moles of reagent, liberating one mole of gas; this indicated one active hydrogen and one addition. The methyl ester, prepared through the silver salt, and from the acid chloride, was oily and was not analyzed.

The oxime. This was prepared in the usual way and recrystallized from *n*-butyl alcohol; it formed small plates (m.p., 181° C.) sparingly soluble in hot methyl and ethyl alcohols, but moderately soluble in ether. Analysis: Calcd. for $C_{23}H_{18}O_3N$: N, 3.9%. Found: N, 3.7%.

C. Oxidation

By the method described in the earlier paper, the acid (V) was oxidized by chromic acid in acetic acid at 40° C., 4.6 gm. giving 2.7 gm. of phenyl benzyl diketone. The latter was identified by conversion into the quinoxaline, m.p. 97° C.,—a mixed melting point with a sample at hand was not depressed. The diketone gave a deep violet-brown color with alcoholic ferric chloride. The methoxy acid (B) on similar treatment gave anisic acid (identified by mixed melting point) and phenyl benzyl diketone.

D.



The acid chloride (VII). The acid (V), 4 gm., and 15 cc. of thionyl chloride was refluxed on a water bath for a half hour, and the solvent allowed to evaporate spontaneously. Long silky needles separated. The halide was purified by recrystallizing several times from ether; m.p. 79° C., turning pink. Analysis: Calcd. for $C_{23}H_{17}O_2Cl$: Cl, 9.8%. Found: Cl, 9.5, 9.7%. The acid was recovered unchanged after refluxing a half hour with acetyl chloride (cf. Ref. 1, p. 611).

The acet-anhydride (VIII). A mixture of 2.4 gm. of the chloride, 2.2 gm. of silver acetate and 20 cc. of absolute ether was refluxed for an hour. After filtering from silver salts the anhydride separated in practically a quantitative yield. On recrystallizing from methyl alcohol it formed dense white prisms; m.p., 126° C. Analysis:—Calcd. for $C_{25}H_{20}O_4$: C, 78.1; H, 5.2%. Found: C, 78.1; H, 5.2%. The same anhydride was also prepared by warming for five minutes 1 gm. of the acid (V) in 10 cc. of acetic anhydride containing a trace of sulphuric acid; it crystallized out after standing a half hour. The acid was regenerated on hydrolysis.

The p-bromanilide (IX). The chloride (2 gm.) and two equivalents (2 gm.) of *p*-bromaniline in 35 cc. of benzene was refluxed for an hour and filtered from the separated *p*-bromaniline hydrochloride. The anilide that remained on partial removal of the solvent was recrystallized to constant melting point from acetone. It formed microscopic needles; m.p., 179° C. Analyses:—Calcd. for $C_{20}H_{22}O_2NBr$: N, 2.8; Br, 16.1%. Found: N, 2.6; Br, 16.3%. The *p*-bromanilide was at first recrystallized from benzene, but it separated with solvent of crystallization. It is insoluble in methyl and ethyl alcohols and cyclohexane, moderately soluble in *n*-propyl alcohol, but easily dissolves in acetone and benzene.

The *p*-iodoanilide was made in essentially the same way, and purified by dissolving in hot benzene and adding an equal volume of cyclohexane; m.p., 200° C. It also separated with benzene of recrystallization. Analysis:—Calcd. for $C_{20}H_{22}O_2NI \cdot C_6H_6$; I, 20.5%. Found: I, 20.7%. Both the anilides gave a red-violet color with concentrated sulphuric acid.

E. Pyrolysis

The acid (V), 137 gm., in a 250-cc. Claisen flask was heated at 230-240° C. for 2½ hr. and the resulting oil distilled *in vacuo*: 114 gm. (95.6% of the theoretical amount) of clear yellow oil was obtained; b.p., 238-250° at 6 mm. After several days it began to solidify. A few crystals were removed for seeding and the remainder of oil and solid dissolved in a minimum amount of warm methyl alcohol; on inoculating with the sample the whole slowly crystallized. When pure it formed white prisms; m.p., 48° C. It is very soluble in the usual organic solvents except petroleum ether but does not crystallize well from any except methyl alcohol. Analysis:—Calcd. for $C_{22}H_{18}O$; C, 88.6; H, 6.0%. Found: C, 88.2, 88.2; H, 6.0, 5.9%. Unless very pure it slowly hydrolyzes, becoming very oily and forming benzaldehyde and benzoic acid. In the Grignard machine it evolved no gas but consumed one mole of reagent, indicating one addition.

The oxime and phenylhydrazone were oils. The 2, 4-dinitrophenylhydrazone, prepared in the usual way, formed carmine needles from chloroform-methyl alcohol solution; m.p., 189° C. Analysis:—Calcd. for $C_{23}H_{22}O_4N_4$: N, 11.7%; Found: N, 11.1%.

Permanganate oxidation of the ketone gave practically the calculated amount of benzoic acid. Chromic acid gave mostly benzoic acid but also

a little oil that probably contained phenyl benzyl diketone, since it gave the brown-violet color with ferric chloride. After ozonization (ozone destroys the diketone) of the pyrolysis product benzaldehyde was obtained, separated by steam distillation and identified by preparation of the phenylhydrazone; m.p., 155° C.

The methoxyketone was obtained only as a thick, viscous, colored oil that had not crystallized after two years; on ozonization as above it gave anisaldehyde. The residual oil gave a ferric chloride test for phenyl benzyl diketone but none could be isolated. The methoxyketone gave an oily 2, 4-dinitrophenylhydrazone.

α -Benzyl- β -chloro- β -phenylpropiophenone (III). (a) A solution of 2 gm. of the unsaturated ketone (IV) in 10 cc. of absolute methyl alcohol was cooled in a freezing mixture while being saturated with hydrogen chloride. After standing in a cold place for two days the white solid that had separated was filtered. It melted over a range, 101-105° C., and was obviously a mixture. After many tedious and fruitless attempts to separate the isomers by fractional crystallization and precipitation from ordinary solvents, a solution in cyclohexane was allowed to evaporate to dryness very slowly. Two kinds of crystals were evident; the larger ones were picked out by hand and when purified formed long narrow plates; m.p., 126° C. The smaller crystals left were then recrystallized several times from methyl alcohol and the stereoisomer obtained in the form of fine needles; m.p., 132° C. Analyses:—Calcd. for $C_{29}H_{19}OCl$; Cl, 10.6%. Found: (126° C.) Cl, 10.4; (132° C.) Cl, 10.4%. The least soluble component of solutions of the mixed chlorides is a mixture that melts at 108° C. and after this has separated very little material remains. The chlorides are insoluble in petroleum ether, slightly soluble in the alcohols and cyclohexane, moderately soluble in ether, and very soluble in chloroform, acetone, and benzene.

(b) *Synthesis.* A mixture of the stereoisomers was prepared by the method of Kohler and Nygaard (4) for a similar chloroketone, from benzaldehyde, benzylacetophenone and hydrogen chloride. The solid obtained was triturated with ether and filtered; 15 gm. of the ketone gave 13.5 gm. of the mixed chloroketones, which was separated as above and found to be identical in crystal form, melting point, and mixed melting point.

Acknowledgment

This work has been assisted by a generous grant from the Cyrus M. Warren Fund of the American Academy of Arts and Sciences.

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NOTE: In the earlier paper (1, p. 612) the melting point of the methyl ether was accidentally omitted. It is 78° C., and the melting point when mixed with the isomeric methyl ester is 58-63° C.

THE ALKALOIDS OF FUMARACEOUS PLANTS

III. A NEW ALKALOID, BICUGULLINE, AND ITS CONSTITUTION¹

BY RICHARD H. F. MANSKE²

Abstract

A base, now named bicuculline, previously isolated from *Dicentra cucullaria* and referred to as alkaloid α has been obtained from *Corydalis sempervirens* and *Adlumia fungosa*. A chemical examination of this alkaloid has disclosed an exceptionally close relation to hydrastine, from which it differs only by the substitution of a methylenedioxy group for the two methoxyl groups. Accordingly, hydrolytic oxidation yields hydrastinine and 2-carboxy-3:4-methylenedioxy-benzaldehyde, which on reduction is converted into the known 3:4-methylenedioxy-phthalide.

In a previous communication, the author (5) recorded the isolation of a base, $C_{20}H_{17}O_6N$ from *D. cucullaria*, which was referred to as alkaloid α . The quantity obtained at that time was insufficient for a detailed chemical investigation, but recently a further supply has become available in the course of a chemical examination of *Corydalis sempervirens* Pers. and of *Adlumia fungosa* Greene, accounts of which it is proposed to publish in due course.

Since alkaloid α has proved to be a distinct substance, it is proposed to name it *bicuculline*, indicative of its botanical origin, the term *Bicuculla* being used by some botanists for the genus *Dicentra*. In connection with its isolation from *A. fungosa*, it may be pointed out that Schlotterbeck (7) obtained from the same plant (*A. cirrhosa* Raf.) a small amount of an alkaloid melting at 176-177° C. Although no analyses are recorded, there can be little question of identity, and the same is probably true of an alkaloid (m.p. 175° C.), regarded as phenolic, isolated from *Corydalis decumbens* by Asahina and Motigase (1) and also not analyzed. It is furthermore possible that bicuculline may have been isolated from other sources without having been recognized as such, on account of a well-defined dimorphism which it exhibits. In one case, it was observed that the base had resolidified in a melting point tube and when the melting point was again determined it had increased from 177° C.* to 193-195° C. At about the same time, an alkaloid melting at 196° C. had been isolated from *A. fungosa*, and inasmuch as bicuculline had been previously isolated from the same plant, identity was immediately suspected.

A supersaturated solution of bicuculline (m.p. 177° C.) in methanol when seeded with a crystal of m.p. 196° C., rapidly deposited the latter form. Furthermore, the hydrochlorides prepared from both forms had the same appearance and melted alone or admixed at 259° C. to a reddish yellow liquid.

Although bicuculline is not precipitated when its solution in acid is poured into an excess of a hot aqueous potassium hydroxide, it was soon recognized that its behavior was more like that of a lactone than that of a phenol. An alcoholic solution of the base when treated with aqueous alkalis, gave an immediate precipitate which dissolved only with considerable difficulty.

If it be assumed that one lactonic group is present, only four more oxygen atoms need be accounted for and, since methoxyl is absent, the presence of

*All melting points are corrected.

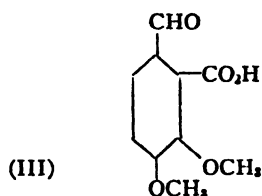
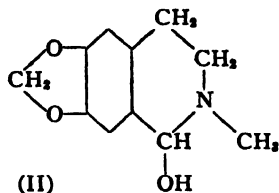
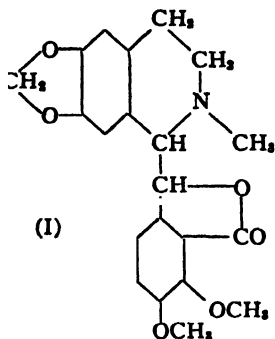
¹ Manuscript received January 16, 1933.

² Contribution from the National Research Laboratories, Ottawa, Canada.

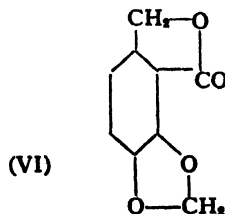
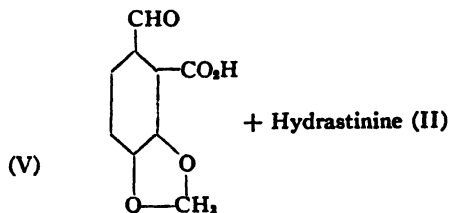
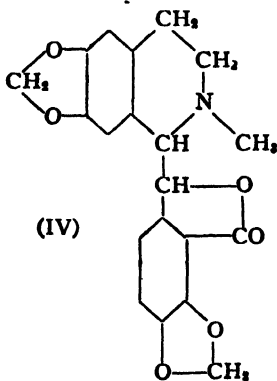
³ Associate Research Chemist, National Research Laboratories, Ottawa.

two methylenedioxy groups would suffice. Finally, if the alkaloid be regarded as an isoquinoline derivative on account of its associates, the conclusion is incumbent that it is closely related to hydrastine, $C_{21}H_{21}O_6N$, from which it differs by CH_4 , *i.e.*, the substitution of a methylenedioxy group for two methoxyl groups. In confirmation of this supposition, it yields the same fluorescent solution that hydrastine gives when heated with manganese dioxide in dilute sulphuric acid.

Recently, Hope, Pyman, Remfry and Robinson (2, p. 244) have described an elegant procedure for the hydrolytic oxidation of hydrastine (I) to yield hydrastinine (II) and opianic acid (III), and when the same conditions are observed in the case of bicuculline (either form) hydrastinine is obtained in nearly quantitative yield.



The acidic fragment was readily isolated from the hydrastinine filtrate, and proved to be an ortho aldehyde acid, treatment with hydroxylamine in hot solution yielding a substituted phthalimide. While these observations yielded no information as to the position of the methylenedioxy group, in the lower nucleus, the precedents of hydrastine and narcotine amply justify formula (IV) for bicuculline.



The aldehydic acid on this basis should have formula (V). This appears to be a new substance, and in fact represents the first example of a methylenedioxy analogue to the well-known opianic acids, attempted syntheses of which have failed (6, 9). Two of the possible methylenedioxy-phthalides are, however, known (6, 9), the substance (VI) having been synthesized by Perkin and Trikojus (6) and later confirmed by Späth and Holter (8, p. 1897). Opianic acid is readily reduced to meconine with sodium amalgam and when the similar reduction was applied to the aldehydic acid from bicuculline, a lactone was obtained which, after repeated recrystallization from water, melted at 234° C. (227° C. uncorr.). It is obviously identical with the substance (VI) which melts at 227° C. (? corr.) (8, p. 1897). The alternative 4 : 5-methylenedioxy-phthalide melting at 189° C. (9) is excluded, so that formula (V) applies to the acidic fragment of the hydrolytic oxidation and consequently formula (IV) completely represents bicuculline.

In conclusion, it may be of interest to point out that alkaloids of the general nuclear structure of hydrastine have been obtained from plants of three different natural orders, namely, Ranunculaceae (hydrastine), Papaveraceae (narcotine), and Fumariaceae (bicuculline), but in each case the phytochemical processes have been subjected to a subtle variation. The analogy, however, goes even further. Both *Hydrastis canadensis* and *Papaver somniferum* contain appreciable amounts of meconine. In the case of *D. cucullaria*, the corresponding methylenedioxy-phthalide (VI) has been also isolated, but whether this is to be regarded as a decomposition product of bicuculline or present as such in the plant is still a matter of doubt.

Experimental

Hydrolytic Oxidation of Bicuculline—Isolation of Hydrastinine (II)

One gram carefully purified bicuculline (m.p. 177° C.) was dissolved in a mixture of 2 cc. of concentrated nitric acid and 8 cc. of water and heated on a steam bath for 20 min. The yellow solution was thoroughly cooled and treated with pellets of potassium hydroxide, one at a time with cooling, until it was strongly alkaline and crystallization of hydrastinine was complete. The solid was filtered off, washed with cold water and dried *in vacuo*. Yield, 0.5 gm. The substance, as thus obtained, was quite pure and melted sharply at 117° C. Treatment in methanol with nitromethane yielded the characteristic addition product, melting sharply at 121-122° C. (uncorr.), first described by Hope and Robinson (3, p. 2136) and stated to melt at the same temperature (? corr.). An aqueous solution of the hydrochloride exhibited an intense greenish-blue fluorescence. With bicuculline of m.p. 196° C. the same products were obtained.

Isolation of 2-Carboxy-3 : 4-methylenedioxy-benzaldehyde (V)

The alkaline filtrate from the hydrastinine was acidified with dilute sulphuric acid and thoroughly extracted with ether. The combined extract was washed with a little water, dried over sodium sulphate and most of the

ether distilled off. The residue was completely freed of solvent and a trace of nitric acid *in vacuo* over potassium hydroxide; yield, 0.5 gm. The crystalline product was dissolved in a little hot water, filtered with the aid of charcoal and cooled, when crystallization readily ensued. The 2-carboxy-3:4-methylenedioxy-benzaldehyde thus obtained melted not quite sharply at 155° C. and gave on gentle warming with phenol and concentrated sulphuric acid, a deep cherry red color which slowly changed to an intense orange.

The dual aldehydic and ortho-carboxylic nature of the substance is shown by the fact that it yields, on treatment with hot alcoholic hydroxylamine acetate, a colorless crystalline substance which is obviously the imide of the corresponding orthodicarboxylic acid. The imide is sparingly soluble in cold water or methanol and when heated in a melting point tube largely sublimes before it melts indefinitely, at about 270° C. When heated with strong alkali on the steam bath, it rapidly dissolves and ammonia is liberated.

Reduction of the Aldehydic Acid to the Phthalide (VI)

An aqueous solution of the substance was acidified with dilute sulphuric acid and treated with sodium amalgam in large excess. When all the sodium had been used up, the aqueous solution was separated from the mercury, freed of a turbidity by means of charcoal and then exhausted with ether. The residue from the ether solution was recrystallized twice from hot water and then consisted of colorless flat elongated plates which melted at 232-233° C. (227° C. uncorr.), and sublimed to the cooler part of the tube. Späth and Holter give the melting point of sublimed 3:4-methylenedioxy-phthalide as 227° C. (? corr.). The substance dissolved readily in warm potassium hydroxide solution. Calcd. for $C_8H_6O_4$: C, 60.67; H, 3.37%. Found: C, 60.48; H, 3.55%.

3:4-Methylenedioxy-N-ethylphthalimide

While the constitution of the aldehydic acid has been sufficiently demonstrated, it seemed nevertheless desirable to approach the matter from another direction. For this purpose, the substance was oxidized in the cold with aqueous potassium permanganate. The filtrate from the manganese dioxide was acidified with sulphuric acid and exhausted with ether. The crystalline residue from the extract was treated with excess ethylamine in aqueous solution, and evaporated to dryness. The residue was distilled *in vacuo* and recrystallized from methanol. It crystallized in colorless needles melting at 130° C. Späth and Holter (8) give 128° C. (? corr.).

N-Methyl-bicuculleine

Since the methohydroxides of hydrastine and of narcotine on appropriate treatment yield substances of which narceine is the best-known representative, it seemed desirable to effect the analogous reaction in the case of bicuculline by Hope and Robinson's (4) method.

One gram of bicuculline in chloroform-methanol was heated under reflux for two hours with an excess of methyl iodide. The solvents were distilled

off and the residue in hot aqueous solution was converted into the methochloride by means of freshly precipitated silver chloride. The colorless filtrate was basified with excess potassium hydroxide in the cold and the orange quaternary base filtered off and washed with cold water. It was then suspended in water and boiled for about 30 min. The undissolved yellow solid was filtered off and boiled again for 30 min. with water. Further treatment failed to change or dissolve this product appreciably. The combined filtrates were evaporated to a small volume. In the course of a short while, colorless plates, melting at 246°C ., crystallized from the cooled solution. The yield was only 0.1 gm. After being twice recrystallized from hot water, N-methylbicumculleine was obtained in fragile colorless plates melting at 250°C . to a brown melt which subsequently effervesced. Calcd. for $\text{C}_{21}\text{H}_{21}\text{O}_7\text{N}$; C, 63.16; H, 5.26; N, 3.51%. Found: C, 62.45; H, 5.40; N, 3.62%.

Bicumculleine and its Hydrochloride

The lower melting form (177°C .) was the first to have been isolated and may be obtained by seeding a cold supersaturated methanolic solution of the base with this form. The higher melting form (196°C .) frequently crystallizes when a concentrated methanolic solution is vigorously boiled. The concentrated methanolic solutions are conveniently prepared by evaporating a chloroform solution of the alkaloid to a resin and dissolving the latter in hot methanol.

Bicumculleine hydrochloride is readily prepared by neutralizing a chloroform solution with alcoholic hydrogen chloride and evaporating repeatedly with chloroform to remove water and alcohol. The cooled concentrated solution generally deposits colorless irregular plates. Cautious addition of ethyl acetate facilitates filtering and washing. The salt melts at 259°C . with but little immediate decomposition. It is readily soluble in hot water and crystallizes from the cooled solution if seeded and particularly if concentrated hydrochloric acid is added.

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INHERITANCE OF BUNT AND LOOSE SMUT REACTION AND OF CERTAIN OTHER CHARACTERS IN KOTA × RED BOBS AND GARNET CROSSES¹

BY THOMAS KILDUFF²

Abstract

Genetic studies were made in F_2 , F_3 and F_4 on the progeny of two common wheat crosses, Kota × Red Bobs and Kota × Garnet. Two chlamydospore collections from pure line hosts were used for inoculation in the bunt studies. The heritable nature of bunt reaction was clearly demonstrated in these crosses and evidence obtained that the degree of resistance found in Garnet must be conditioned by a single major factor. No genic hypothesis could be offered to explain the findings in regard to loose smut reaction due to a lack of agreement in infection percentages in the F_2 and F_4 . Red kernel color in Kota and Red Bobs was found to be governed by two factor pairs. The results on the inheritance of awn characters agreed with the earlier findings of Clark and other workers with crosses of similar awned types, several factor pairs being involved. Two main factors appeared to condition the difference in strength of straw between Kota and Garnet, but the entire expression of the character can best be explained on a multiple factor basis. Correlated inheritance studies, made with the use of the correlation ratio η , indicated the presence of two linkage groups concerned with reaction to the bunt forms used. However, the linkage apparent between strength of straw and bunt susceptibility factors and between awn type and bunt resistance factors is not considered to be close enough to interfere with breeding for desirable bunt resistant varieties.

Introduction

Attention has been drawn recently by Aamodt (1) to the need in western Canada for improved varieties of hard red spring wheat resistant to those diseases which are likely to be destructive and limiting factors in production. The wheat improvement program at the University of Alberta considers not only resistance to bunt, loose smut, foot rots, drought and lodging, but keeps to the fore consideration of such important agronomic characters as earliness, quality and high yielding ability. The program also includes basic genetic studies on wheat plant characters, both morphologic and physiologic. The study here reported was undertaken, as part of the more general program, to ascertain the mode of inheritance of reaction to bunt and loose smut in certain common wheat crosses and to discover if possible any relation existing between disease reaction and the other characters studied, namely, seed color, awning and strength of straw.

Materials

Parental Varieties

Three varieties of hard red spring wheat, *Triticum vulgare*, Host., were used as parents in the two crosses studied. All three, namely, Kota, Red Bobs, and Garnet, are good yielders and fairly well adapted to central Alberta.

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Contribution from the Department of Field Crops, University of Alberta, Edmonton, Canada, with financial assistance from the National Research Council of Canada. This work is in part from a thesis presented to the Committee on Graduate Studies, University of Alberta, in partial fulfilment of the requirements for the degree of Master of Science.

² Formerly Graduate Assistant in Genetics and Plant Breeding, University of Alberta.

Kota was originally introduced into North Dakota from Russia by H. L. Bolley in 1903, but it was not produced on a commercial scale until 1919. It is a midseason variety, moderately drought resistant and resistant to many physiologic forms of *Puccinia graminis tritici* in the field, but it is susceptible to bunt and loose smut. The mid-dense, fusiform head bears strong awns 30 to 80 mm. in length. Not only are there long awns on the lemmas or flowering glumes, but the beak of the outer glume is elongated 3 to 20 mm. to an awnlike point. The straw is somewhat weak and under very favorable growing conditions shows a distinct tendency to lodge (10). Kota is not a good milling and bread-making wheat.

Red Bobs originated from a red-kernelled head selection made in 1910 by Seager Wheeler, Rosthern, Saskatchewan, from a field of White Bobs. It was distributed commercially in 1918. Red Bobs is an early to midseason variety, not particularly drought-resistant and susceptible to stem rust. It proved to be immune to the collection of loose smut used in these experiments. The oblong, mid-dense head is sometimes clavate, is entirely without awns, or awnlets, and has beaks wanting to 0.5 mm. long. The straw of this variety is moderately strong, standing up fairly well under most conditions (10). Red Bobs is a good milling wheat and its flour is nearly equal in quality to that of Marquis.

Garnet, a comparatively recent (1926) entrant to the group of commercially important wheats, is a production of the Central Experimental Farm, Ottawa. It is from a Preston A \times Riga M cross made by Chas. Saunders in 1905. This variety is considerably earlier than Red Bobs, but is also very susceptible to stem rust. In these tests it proved to be resistant to bunt and loose smut. The fusiform mid-dense head shows considerable variability in regard to the length and distribution of the awnlets. Some heads are almost bald, some bear a few short apical awnlets (2 to 5 mm.) only, while the majority have awnlets (2 to 10 mm.) well distributed down the spike. The beaks are about 1 mm. long. The straw is fine and mid-strong, under some conditions showing a tendency toward weakness. Garnet is discriminated against by some millers and bakers on account of the lower quality and the distinctly yellow tinge in the flour it produces (31, 35).

Crosses

In 1929, a number of F_2 bulk crosses were received from J. A. Clark, of the Division of Cereal Crops and Diseases, U.S.D.A., Washington, D.C. Of these, two separate populations of a Kota \times Garnet cross (No. 109 and No. 112) and two of a Kota \times Red Bobs cross (No. 115 and No. 118) were turned over to the writer for the present study.

Bunt Inoculum

Two chlamydospore collections of *Tilletia*, which had been grown for two years (1928-29) on pure hosts in the University varietal plots, were used for inoculating the F_2 plants. The inoculum referred to in this study as "Kota bunt" was obtained from bunted heads of the Kota variety and that as

"Red Bobs bunt" from Red Bobs (University of Alberta No. 222). A chlamydospore collection was taken from the parental check rows of Kota which had been inoculated with Kota bunt in 1930 and was used for inoculating those lines tested in F_4 . The Kota bunt collections used in 1930 and 1931 consisted of *Tilletia levis* Kühn. The Red Bobs bunt used in 1930 was *T. tritici* (Bjerk.) Wint. with a small percentage admixture of *T. levis*. This chlamydospore collection was the same as collection No. 1 used by Aamodt (1). In 1931 the Red Bobs bunt saved from the parental checks was accidentally destroyed. A composite inoculum from bunted heads of the Red Bobs \times Kota F_3 hybrids was used as Red Bobs bunt in the tests of that year. Microscopic examination showed this collection to consist of approximately one-third *T. levis* and two-thirds *T. tritici*.

Loose Smut Inoculum

The inoculum for the study of reaction toward loose smut, caused by *Ustilago tritici* (Pers.) Jens., was obtained in 1929 from the newly emerged sporulating heads rogued from special Kota and Ceres plots, and from Kota parental check rows in breeding plots. In 1930 inoculum was obtained from similar sources and also from F_3 hybrid plants.

Methods

General

The seed from individual F_2 and F_3 plants (F_3 and F_4 lines respectively) was spaced in five-foot rows one foot apart and handled on the individual-plant basis. Parental checks were grown at intervals of 30 rows with each generation and treated in the same manner as the hybrid material.

The F_3 was grown in three series. The first series was grown in a genetic and loose smut nursery. The second was in a bunt nursery where the seed was inoculated with chlamydospores from Kota, and the third, when there was sufficient seed, in a bunt nursery where the seed was inoculated with chlamydospores from Red Bobs.

Bunt Inoculation

The bunted heads from the sources already described were passed through a small hand grinder that reduced the whole to a fine powder. Care was taken to avoid contamination of each lot by spores from the other. The inoculum was applied to the seed by shaking well a small portion of the ground chlamydospores in the envelope with the seed. The quantity used was approximately 1 part of inoculum to 25 of seed by weight.

In the taking of bunt data, counts were made in the field at maturity. Each head was carefully examined, and a plant was considered to be infected if one or more florets on any head proved to be bunted.

Only those F_3 lines showing a combination of earliness, good strength of straw, and a degree of bunt resistance similar to Garnet were tested in F_4 for reaction to Kota bunt and Red Bobs bunt. These F_4 tests were made

on the progeny of six plants from each selected line grown in short 25-seed rows, and a further test made on a 50-seed row inoculated with a composite of the two bunt collections.

Loose Smut Inoculation

The F_2 plants were inoculated with loose smut by brushing the wheat heads with a small brush of 10 to 20 infected spikes. This was done three times at three-day intervals during the flowering period of the hybrids and parental check plants. When each treatment was complete the spikes were covered with a brown smudge of spores. The weather during this period was clear and dry.

A minimum of five heads from as many plants in each F_3 line of population No. 109 of the Kota \times Garnet cross were inoculated by Stringfield's method (48). Spaced plant-rows of 20 to 30 seeds each of this material were grown in 1931 to check in F_4 the F_3 findings. In a preliminary test in the greenhouse this inoculation method yielded as high as 75% infection.

Bunt

Considerable literature dealing with breeding and inheritance studies on wheat in relation to bunt reaction has been well reviewed by Gaines (13), Briggs (3, 4) and more recently by Aamodt (1). Consequently mention need only be made in this paper of those authors whose works have a direct bearing on the results reported herein.

The population of many of the F_3 lines tested for bunt reaction was reduced to a few plants by cutworms and a prolonged period of soil drifting in the spring of 1930. This was particularly true of lines located at the western and northern limits of the bunt nursery. However, since the distribution of the lines was essentially on a random basis in the seeding plan, a reduction in the amount of data from which to draw conclusions was the only appreciable effect of this damage. In summarizing the data, all lines containing less than ten plants were disregarded, the amount of infection was expressed on a percentage basis, and the lines were grouped in classes according to the percentage of bunted plants. A class interval of 10% was used, the bunt classes being described as 5, 15, 25, etc., denoting the infections within the ranges from 1 to 9%, 10 to 19%, 20 to 29%, etc., respectively. A zero class was added to include all lines free from bunt.

In addition, the percentage infection of the entire parental population used as checks was computed and is shown in Table I. This seemed advisable since only two rows of the Red Bobs checks in the Red Bobs bunt series contained the minimum number of ten plants required for the method of summarization just described.

The lower percentage of infection obtained in 1930 in all three varieties inoculated with Red Bobs bunt might be interpreted as due to a weakness in the infection capacity of that particular lot of bunt, lack of maturity, or artificial injury to the sample, rather than to its inherent infection capabilities. However, in view of the difference in behavior of the two lots of bunt on

Kota and Red Bobs, it must be concluded that the original collections consisted essentially of differing physiologic forms or groups of forms. The virulence of the original Red Bobs bunt appeared to be considerably less than that of the Kota bunt. The reaction of the three varieties to Kota bunt was substantially the same in both years. This cannot be said of the Red Bobs collections where a like reaction was hardly to be expected in view of the different sources of origin and the differences in species composition as revealed by the microscope.

TABLE I
AVERAGE PER CENT SMUT-INFECTED PLANTS OF PARENTAL VARIETIES

Smut	Variety					
	Kota		Red Bobs		Garnet	
	1930	1931	1930	1931	1930	1931
Kota bunt	68.3	54.8	51.2	51.8	10.4	8.1
Red Bobs bunt	30.0	68.4	36.0	51.6	2.6	9.7
Loose smut	1.5	20.4	0.0	—	0.6	5.1

At the beginning of this study the two populations in each cross were considered separately. The results for the two populations proved to be so similar that it was decided to bulk the data. This decision was based, in part, on the data presented in Table II. These data may be taken to indicate not only the identity of the duplicate populations but also the uniformity of the conditions of test, and they bear out the assumption made in regard to the randomness of sampling in the material included for study.

TABLE II
DISTRIBUTION INTO INFECTION CLASSES OF F_2 LINES OF KOTA \times RED BOBS AND KOTA \times RED BOBS AND KOTA \times GARNET HYBRID POPULATIONS WHEN INOCULATED WITH TWO COLLECTIONS OF BUNT

Cross	Kind of inoculum	Popula- tion No.	Numbers in percentage infection classes												Total
			0	5	15	25	35	45	55	65	75	85	95		
Kota X Red Bobs	Kota bunt	115				1	3	15	28	34	32	20	10	143	
		118				5	15	22	26	33	34	8	2	145	
Kota X Red Bobs	Red Bobs bunt	115		1	7	13	11	16	7	9	3	1		68	
		118	2	7	10	11	23	17	9	7	3			89	
Kota X Garnet	Kota bunt	109	2	6	7	13	28	27	12	19	5	4	1	124	
		112	1	6	11	22	29	22	13	10	8	2	1	125	
Kota X Garnet	Red Bobs bunt	109	57	32	20	7	3	1		1				121	
		112	30	35	23	9	9	5	2					113	

A study was made of the distribution in the infection classes of the lines that had ten or more plants in both bunt tests. As this reduced number of strictly comparable lines followed very closely in distribution the total population in each cross it was decided to use the larger numbers.

The data in Table III show very well the heritable nature of bunt reaction. In the susceptible \times susceptible crosses (Kota \times Red Bobs) the hybrids occupy the entire parental infection range and no segregates more resistant than either parent appear. The majority of lines fall in the classes showing 50 to 80% infection. These results are in agreement with those obtained by Briggs (3) in Baart \times Hard Federation crosses.

TABLE III

DISTRIBUTION INTO INFECTION CLASSES OF PARENTAL VARIETY ROWS AND F_2 LINES WHEN INOCULATED WITH BUNT FROM KOTA (SERIES 2)

Parents and crosses	Numbers in percentage infection classes											Total of lines
	0	5	15	25	35	45	55	65	75	85	95	
Kota	—	—	—	—	1	4	—	5	4	4	2	20
Red Bobs	—	—	—	2	—	2	3	2	—	—	—	9
Garnet	2	2	5	—	—	—	—	—	—	—	—	9
Kota \times Red Bobs	—	—	—	6	18	37	54	67	66	28	12	288
Kota \times Garnet	3	12	18	35	57	49	25	29	13	6	2	234

The Kota \times Garnet segregation shows distinctly the introduction into the hybrids of factors resistant to Kota bunt. In discussing hybrids of which Garnet is a parent it must be remembered that the resistance of this variety is much greater in degree than the number of infected plants would indicate. Bunted Garnet plants seldom showed more than one partly bunted head, whereas bunted Kota and Red Bobs plants usually showed several. In some cases every culm of these varieties bore a bunted head. A considerable number of the hybrids showed resistance equal to that of the Garnet parent, a larger number exhibited the high susceptibility of the Kota parent, but the majority fell in the classes showing 20 to 50% of the plants bunted.

TABLE IV

DISTRIBUTION INTO INFECTION CLASSES OF PARENTAL VARIETY ROWS AND F_2 LINES WHEN INOCULATED WITH BUNT FROM RED BOBS (SERIES 3)

Parents and crosses	Numbers in percentage infection classes											Total
	0	5	15	25	35	45	55	65	75	85	95	
Kota	—	—	6	7	2	2	3	1	—	—	—	21
Red Bobs	—	—	1	—	—	—	1	—	—	—	—	2
Garnet	6	3	—	—	—	—	—	—	—	—	—	9
Kota \times Red Bobs	2	8	17	24	34	33	16	16	6	1	—	157
Kota \times Garnet	87	67	43	16	12	6	2	1	—	—	—	234

The data in Table IV show that the behavior of the Kota \times Red Bobs hybrids toward Red Bobs bunt resembles their reaction toward Kota bunt, except that the whole distribution is moved toward the lower infection classes. The Kota \times Garnet hybrids show a considerable aggregation in the non-bunted class and the two lowest infection classes.

Loose Smut

There is a dearth of literature on inheritance of resistance to loose smut of wheat, probably because no rapid and effective method of inoculating the wheat flower has been devised. The laborious method of opening and inoculating each flower by hand during the short period when infection can take place probably has been the factor limiting such studies.

Olson, *et al.*, (40) in 1920, were the first to report on inheritance of resistance to this smut. A review of their work by Matsuura (33) states, "there are several genes involved for resistance to smut caused by *Ustilago tritici* in different wheat varieties". These are said to be transmitted independently and to have a cumulative effect when they act together. No mention is made of the methods used in arriving at these conclusions.

Tisdale and Tapke (51) pointed out in 1927, as a result of inoculation studies, that highly resistant and immune strains were found in many varieties of common wheat. Fultz, Fulcaster, Hussar, and Ridit, winter varieties, and Preston, a hard red spring wheat, each contained such strains.

Later extensive tests by Tapke (49) supplemented this list of varieties and confirmed the observations on field resistance in certain varieties by showing these same varieties to be resistant following artificial hand-inoculation. On the whole the club wheats tested were found to be very susceptible and Kota, a common wheat, showed the same degree of susceptibility. The use of composite samples of spores, instead of spores from single heads, in the latter years of this test showed that some varieties, thought to be resistant or immune, were highly susceptible. The presence of physiologic forms of the pathogene was demonstrated by this change in the reaction of certain varieties.

In a test of six years duration on the continued selection of large and small seed, an increase in amount of loose smut in the plots sown to small seeds was noted by Taylor (50). He suggested that this was probably due to some effect of the loose smut organism within the developing kernel on endosperm development and not to any heritable factors.

In a study which demonstrated that *Ustilago tritici* possessed physiologic strains Piekenbrock (43) made resistance and susceptibility tests on many European varieties and hybrids. Later, crosses were made between a highly resistant variety, Grüne Dame, and two susceptible wheats, Rumker's Dickkopf and Rimpau's Schlanstedter. Segregation was such as to indicate that immunity was inherited as a recessive. This author describes an inoculation mechanism and technique that would appear to be an improvement on the usual methods.

Grevel (17), another German worker, confirmed Piekenbrock's conclusions that resistance to loose smut in wheat is inherited as a Mendelian recessive. Neither of these workers, however, was able to formulate a simple factorial explanation. Grevel obtained strains from two crosses of resistant \times susceptible varieties that were immune from biotypes I and II of the four physiological strains he has established in *U. tritici*.

Preliminary tests made in the greenhouse during the winter months of 1929-1930 indicated that the percentage of infection obtained from inoculating with loose smut was very low. This was borne out later by the field studies. Only three of the lines showing infection in the genetic series were infected in the two bunt series. In view of this condition it was decided to make no distinction among the three series, but to summarize the results on the basis of the three replicates as one. Only a few lines had a total of less than 25 plants. A line was considered to be infected when one or more heads or parts of heads on any plant produced loose smut. Few lines showed more than one or two infected plants. The data are given in Table V.

TABLE V

LOOSE SMUT INFECTION OF PARENTS AND F_3 HYBRID LINES FROM KOTA \times RED BOBS AND KOTA \times GARNET CROSSES

Parents and crosses	Total lines	Number of lines infected	Percentage of lines infected
Kota	26	12	46.2
Red Bobs	15	0	0.0
Garnet	12	3	25.0
Kota \times Red Bobs (115)	200	40	20.0
Kota \times Red Bobs (118)	195	42	21.5
Kota \times Garnet (109)	164	69	42.1
Kota \times Garnet (112)	179	73	40.8

The reaction of the hybrids is in agreement with that of the parents in each cross. Forty-six per cent of the Kota checks were infected, 25% of the Garnet checks and 42.1% and 40.8% in two separate populations of the F_3 Kota \times Garnet hybrids. The two Kota \times Red Bobs populations had 20 to 21.5% of the F_3 lines infected, showing an intermediate condition between the 46.2% infection of the Kota parent and the immunity of the Red Bobs parent. The close check between similar populations in the same cross with regard to the percentage of lines showing infection is of interest in view of the low degree of infection obtained, and is further evidence that the two populations are similar for factors governing the reaction to loose smut.

TABLE VI

SUMMARY OF REACTION OF KOTA \times GARNET F_3 AND F_4 LINES (POPULATION No. 109) TO *U. Tritici*

F_3 reaction (1930)		F_4 reaction (1931)		
Infection	Number of lines	No infection	Some rows infected	All rows infected
None	53	3	38	12
Some	37	4	23	10
Totals	90	7	61	22

One hundred and sixty-four F_4 lines were sown in 1931. Owing to heavy rains in midsummer 74 of these were drowned out. Of the 90 remaining, 53 lines which had shown no infection in 1930 gave the following reaction in 1931: 3 continued to show no infection, in 38 some rows showed infection and in 12 all rows were infected. The infected rows in the last two classes had an average infection rate of 12 and 24% respectively. Of the 37 lines which had shown some infection in 1930, 4 showed no infection, in 23 some rows were infected, and in 10 all rows were infected. The infection percentages in infected rows in the last two classes were 12 and 20% respectively. These data are summarized in Table VI and demonstrate that there is not sufficient correlation between the infection in the two generations to make a genetic analysis. This may be due in part to the inadequacy of the 1930 tests, as a result of faulty inoculation technique, or to the possible presence of physiologic forms of the pathogene in the inoculum.

Consideration of the data presented in Table I would appear to indicate that a certain amount of resistance to loose smut must be present in Garnet. Of the 17 check rows of Kota 14 showed infection, which on a plant basis gave an infection rate of 20.4%. On the other hand only 9 out of the 22 Garnet check rows showed infection and the infection rate on a plant basis was 5.1%. It may be that both parents are similar in physiological susceptibility to the inoculum used but there may exist some functional difference in period of glume opening, in degree of glume opening or in the length of time glumes remain open during the flowering period. Any of these factors would vary the opportunity of obtaining infection under the conditions of test. Such phenomena would presumably be characteristic in a given variety and would explain the constant reaction of the parental varieties as well as the perfectly random manner in which the hybrids were affected in these tests.

Seed Color

Since seed color is a striking characteristic of any wheat variety, and of considerable economic importance, it is not surprising that it was one of the first characters genetically investigated in the wheat species. Biffen (2) was the first to report on the inheritance of red kernel color in wheat. He found red to be dominant over white in F_1 and to segregate on a single factor basis in F_2 .

The studies of Nilsson-Ehle (36, 37, 38, 39) on color inheritance in wheat established both two- and three-factor hypotheses and laid the foundation for the present conception of the inheritance of quantitative characters. An analysis of his F_3 results led him to postulate that the red grain color of the "Sammet" parent was due to the presence of three dominant and independent factors, R_1 , R_2 and R_3 , each of which by itself was capable of producing redness. He explained the varying shades of red obtained as due to "additive or cumulative action of the several color factors", the presence of two or three factors giving twice or thrice the depth of color that one factor would produce. Only when the three factor pairs were homozygous for red would

the original dark red of the red parent be recovered. The whites would all breed true since they must be homozygous recessive, and between these extremes would be five shades of red. Intermediate types of red could not be readily separated on the basis of color intensity alone and recourse was made to classification on the basis of breeding behavior. Nilsson-Ehle was the first to record the occurrence of white grained plants in the F_2 from a red \times red cross (39).

Howard and Howard (28, 29), working about the same time as Nilsson-Ehle, published in 1912 and 1915 evidence of a similar nature showing the existence of two factors for red in some Indian varieties of *T. vulgare*, and the presence of three factors for red in a *T. compactum* known as "American Club".

In a series of crosses in Washington, 1917, between several club varieties and several common wheats, Gaines (12) confirmed the two- and three-factor hypothesis of Nilsson-Ehle. In analyzing his results he used only three red color classes. He assumes that each factor for R produces the same color of red whether heterozygous or homozygous, and that the effect of the different factors R, R', and R'' is cumulative. These assumptions differ but little from those of Nilsson-Ehle, the differences being due to the difficulty in distinguishing between the shades of color.

On the whole, Gaines' results support the assumption of three independent factors working together to produce the red and white color phenomena in wheats. He points out, however, that it is not sufficient to explain all the color inheritance results found. He reports two cases in which red seed was produced from a white \times white cross. This would indicate that another factor or set of factors was concerned, which would probably be inhibitory in effect.

Percival (42) states that the usual result of crossing two white-grained wheats is the production in subsequent generations of white-grained plants only. He reports, however, that two workers found otherwise. Pitsch and Vilmorin obtained red-grained plants in different crosses in which both parents were white-grained. These results, with a few discrepancies to be noted in some of Nilsson-Ehle's work, lend support to the possibility of some inhibitory factor or set of factors operating in color inheritance.

Since these earlier investigations many others have been undertaken. Harrington (19) studied some of our common wheats and concluded that Kitchener contained two independently inherited factors for red grain color. In some strains of Red Bobs and Hard Red Calcutta he found two factors, in others one. This probably indicates that these two varieties are made up of red strains that are indistinguishable, one of which has a single factor pair while the other has two pairs for red. Awning and seed color were studied in these crosses and were found to be independently inherited.

Harrington and Aamodt (22) found no relation between seed color and resistance to *Puccinia graminis tritici* but report a 3 : 1 ratio for red and white color in the F_2 of the durum crosses they studied.

Clark (6) reports on several reciprocal crosses between Hard Federation and Kota. He obtained a fairly good agreement with a theoretical two-factor ratio. When Kota was used as the female parent a much better fit was obtained than when the reciprocal cross was made, suggesting a possible slight maternal influence.

Hayes and Robertson (27) worked with red \times white crosses using Bobs as the white parent. The Marquis \times Bobs crosses, made reciprocally, gave a 15 : 1 ratio indicating that Marquis carries two independent factors for red. The red \times red crosses gave a 63 : 1 ratio for red and white in F_2 . The results were explained on the basis of two factors for red in Marquis and a single but different factor in Minturki and Kanred, the other varieties used. In one cross the "Marquis" parent was proved to be Red Fife, and, since no disturbance of the expected two-factor ratio occurred, it may be surmised that Red Fife has a complement of color factors similar to that of Marquis.

Clark and Hooker (9), like all former workers, found red to be dominant over white. They used reciprocal crosses of Marquis and Hard Federation, and in 10 families out of 12 studied, obtained a very good fit for a 15 : 1 ratio, indicating that two factors are concerned with red kernel color in Marquis. The two other families gave a satisfactory fit for a 3 : 1 ratio indicating that a single factor only was concerned. This difference in the ratios obtained indicates, according to Clark and Hooker, that either Marquis does not have homozygous dominant factors for red-kernel color, or the Hard Federation parents differed in their genotypes.

Stewart (45) has recently obtained segregation on a three-factor basis in a pure line red \times white cross, using Kanred as the red parent. Correction of the F_2 analysis was made in F_3 and, although an excess of true-breeding reds and a deficiency in the segregating classes was found, this was explained by the fact that only 30 to 40 plants in each progeny were available. Such numbers were not thought to be sufficient to show the one plant with white grains where a 63 : 1 segregation was expected. Stewart's findings are not in accord with those of Hayes and Robertson (27) who found only one factor for red in Kanred.

Stewart and Tingey (47) found duplicate factors for red color in the cross Marquis \times Federation (red \times white). An attempt was made to correlate glume and seed color factors but these proved to be inherited independently. When a light-red kernelled variety, Odessa, was used as one parent in a red \times white cross, Stewart and Price (46) found a single factor difference responsible for color segregation.

Matsuura (33) reports that several European investigators working with various species and varieties also found mono-, di- and trigenic ratios in color inheritance.

In the work reported here, only the Kota \times Red Bobs crosses were studied for seed color, since no segregation for this character took place in the Kota \times Garnet crosses. The seed from the F_2 plants was classified as either red

or white. In F_2 , twenty-five plants were taken on which to determine color segregation in each line. Since there were seldom twenty-five plants available in the genetic series rows, the deficiency was supplied by plants taken at random from the bunt-free population in replicates of the Kota bunt and Red Bobs bunt series. Seed color determinations were made as in F_2 by ocular examination.

The segregation for seed color noted in the Kota \times Red Bobs F_2 hybrids clearly indicated that a two-factor difference for color exists in this cross. Table VII shows the F_2 results. The odds of 1.31 : 1 indicate a very good fit.

TABLE VII
SEGREGATION IN F_2 FOR SEED COLOR IN KOTA \times RED BOBS CROSSES

Color class	Number		Dev.	P.E.*	$\frac{D.}{P.E.}$	Odds
	Observed	Calculated				
Red	376	372.2	3.8	3.25	1.16	1.31 : 1
White	21	24.8				

* Probable errors for numbers of individuals given here and elsewhere in this paper were obtained from tables of probable errors of Mendelian ratios, prepared by the Department of Plant Breeding, Cornell University, Ithaca, N.Y.

A population segregating on a 15 red : 1 white basis in F_2 , is expected to give a 7 : 4 : 4 : 1 ratio in F_3 . That this expectation was not fulfilled in this case is shown by the data in Table VIII.

TABLE VIII
SEGREGATION IN F_3 FOR SEED COLOR IN KOTA \times RED BOBS HYBRIDS

Color class	Expected ratio	Number		O-C	(O-C) ²	$\frac{(O-C)^2}{C}$
		Observed	Calculated			
Red	7	324	168.9	155.1	24056.0	142.43
15R : 1W	4	15	96.5	81.5	6642.3	68.83
3R : 1W	4	26	96.5	70.5	4970.3	51.51
White	1	21	24.1	3.1	9.6	0.40

P = extremely small

$X^2 = 263.17$

The fit for the segregating classes and for the true-breeding red class is very poor. The discrepancy may be due in part to the difficulty in distinguishing, in vitreous grain samples, between very pale red types and those having no pigmentation; or to the classifying of some white samples as pale red as a result of their discoloration by disease. The most probable cause, however, may be the small numbers used to test segregation in F_3 . In most cases 25 plants in each line were used for this purpose. It is believed that

many lines assigned to the apparently true-breeding red class would have shown segregation if larger numbers had been employed. This explanation was advanced by Stewart (45) to explain a similar deficiency in his segregating classes.

Since a two-factor difference for color is indicated in this red \times red cross it would appear that Red Bobs and Kota each carry one factor pair for red which is unlike that carried by the other. Harrington (19) found two factors for color in some strains of Red Bobs and one factor in others. Clark and Hooker (9) have reported the same condition to hold for Marquis. An even more striking contrast exists in the finding of one factor for red by Hayes and Robertson (27) and three factors by Stewart (45) in the variety Kanred. The findings here reported are at variance with those of Clark (6) who found seed color in Kota to be governed by two factors. On the whole it would appear that many commonly grown varieties of wheat are not homogeneous for color. Lack of homogeneity within these varieties doubtless exists in relation to other characters and its demonstration serves to emphasize the presence and probable importance of strains and pure lines in the common varieties.

AWNS

The literature on inheritance of awns is quite voluminous and much confusion appears in the early studies due to inexact and faulty classification of awn types. All lines not fully awned were usually considered as awnless and nearly all results were interpreted on the basis of a single partially dominant factor for awns. This early work is well summarized by Percival (42), Kezer and Boyack (30), and Gaines (12). They reported a single factor difference for awning in the varieties studied but disagree in regard to the partial dominance of awning or awnlessness. The Howards (28, 29) were the first to recognize differences in the so-called awnless groups. They established the presence of two factors controlling the length of tip awns within that class. Harrington (19) used their method of awn classification and from appropriate crosses obtained both single factor and two factor results.

At present two systems of awn classification are in common use. One, essentially the same as that used by the Howards, is now used by most American investigators. A description of the five awn classes employed in this system given by Clark (6), follows:—

“(1) awnless, (2) apically-awnletted, (3) awnletted, (4) short-awned, and (5) awned. Class I, awnless, normally is entirely without awnlets in the apical part of the spike, although a few awnlets 1 to 2 mm. long may occur at the apex under abnormal conditions. Class 2, apically-awnletted, has awnlets 2 to 20 mm. long at the apex of the spike but rarely extending to the central and basal portions. Class 3, awnletted, has awnlets from 3 to 40 mm. long, the shorter occurring at the base of the spike and the length increasing toward the apex. Class 4, short-awned, has short awns throughout, varying from 15 to 50 mm. long but only about half the length of the normal awns. In Class 5, awned, the awns vary from 30 to 80 mm. in length.”

Clark found that these classes were fairly definite and that any doubt regarding classification would be cleared up by studying the breeding behavior in F_3 . The other system which involves only four true-breeding awn classes, was described by Stewart (44). Although considerable difficulty is said to be encountered in classifying intermediate types in F_2 , a study of the F_3 breeding behavior makes for accuracy and the method is considered adequate by Stewart and coworkers (45, 46, 47). A description of the classes follows:—

"Class 1, awnless, or almost so; class 2, short-tip awns; class 3, short-tip awns in lower half of spike and part-length awns in upper half; and class 4, fully awned."

The detailed findings of the various investigators in regard to the numerous crosses studied for the inheritance of awning will not be recounted here. However, a few of the results obtained from linkage studies may be mentioned briefly. The importance of a possible linkage between awn factors and factors governing resistance or susceptibility to disease is obvious. So also is any association that may be found between awns and yield, quality of grain, or other agronomic quality. Although numerous tests for linkage have been made in awn studies to date no linkage has been reported between awn factors and factors for either rust or smut reaction (14, 16, 21, 34). In regard to yield and protein content some indications of relation exist. Hayes (24), after studying crosses of Marquis on Preston and Bluestem, concluded that "the bearded families average somewhat higher than the awnless families for average length of seed, average percentage of plumpness of seed, and average yield per plant". Later, he and coworkers (26) found that the awned lines of spring and winter wheats, on the average, excelled in plumpness of grain and that a high degree of correlation existed between plumpness of grain and yield. Clark and coworkers in a series of studies (6, 7, 8, 11) found varying degrees of direct relation between length of awn and crude protein content, and noted in all except one case a positive correlation between awn length and yield. In the exceptional case (11) opposite results were obtained.

The two crosses described in foregoing sections were studied in F_2 and F_3 for segregation of awn characters. The five awn classes of Clark (6), already described in the literature review, were used as a basis of classification in this study.

In F_2 an attempt was made to place each plant in one of the five classes. In addition, when the plant did not appear to fit the centre of the class range, an estimate was recorded by the use of plus and minus signs to indicate whether the plant approached the upper or lower limit of the class. Also an average measurement, taken in millimetres, was recorded for the awn and beak. These measurements were taken near the centre of the awn or awnlet range and consisted of four readings on from two to four well-developed heads.

The F_3 lines were classified plant by plant, no attempt being made to make any finer classification than that provided by Clark's five awn classes. At this stage the three replicates were checked against each other to eliminate

any errors that might have crept in during the handling and preparation of the seed or in seeding. Thus a better judgment of the purity of the individual lines was obtained and data for material damaged while in the stook were readily corrected. Except in cases where such damage occurred, awn classification was made on the material grown in the genetic series.

The F_2 segregation for awns in the Kota \times Red Bobs crosses agreed very closely with the findings of Clark (6) in some Kota \times Hard Federation crosses. His grouping of the awnless classes 1, 2, and 3, and the awned classes 4 and 5, gave a 3 : 1 ratio with odds of 1.46 : 1, indicating a close fit. A similar grouping of the segregating F_2 Kota \times Red Bobs hybrids is shown in Table IX. The F_2 classification has been corrected by the breeding behavior of F_3 in these data. The odds of 2.46 : 1 against the occurrence, due entirely to chance, of a deviation as great or greater than the one observed, indicates a good fit to the theoretical ratio.

TABLE IX

SEGREGATION FOR AWNING IN KOTA \times RED BOBS CROSSES IN F_2 , AND CALCULATION OF GOODNESS OF FIT TO A 3 : 1 RATIO

Awn class	Numbers		Dev.	P.E.	D. P.E.	Odds
	Observed	Calculated				
1 2 3	49 108 140 } 297	288	9	5.72	1.57	2.46 : 1
4 5	14 73 } 87	96				

Awn class 1—awnless; Awn class 2—apically-awnletted; Awn class 3—awnletted; Awn class 4—short awned; Awn class 5—awned.

While the presence of one major factor difference is indicated by these and by Clark's results, it is probably concerned principally with the presence or absence of the long, strong Kota awn. The presence of true-breeding lines in classes 2 and 3 requires a two- or multiple-factor hypothesis. Unless a detailed analysis were made of the breeding behavior of the F_3 segregates in F_4 it would be impossible to say more than that in these crosses, two or more factors for awns must be involved, and that the segregation already obtained resembles the results of Clark and others (6, 8) in crosses of a similar nature.

In the Garnet \times Kota hybrids the segregating F_2 population could be grouped, on the basis of F_3 breeding behavior, into three classes. These were awn classes 3 and 5, and a segregating class. Although several F_2 plants had been placed in class 4, all of these were observed to segregate in F_3 . Ten plants, classified as class 2 in F_2 , were included in class 3 as were the few lines segregating for classes 2 and 3. The findings of other workers

TABLE X

SEGREGATION FOR AWNING IN KOTA \times GARNET HYBRIDS IN F_2 , AND CALCULATION OF GOODNESS OF FIT TO A 1 : 2 : 1 RATIO

Awn class	Numbers		O - C	(O - C) ²	$\frac{(O - C)^2}{C}$
	Observed	Calculated			
3	58	85.7	27.7	770.1	9.88
Segregating	173	171.5	1.5	2.3	0.01
5	112	85.7	26.3	689.1	8.04

$$P = 0.00013$$

$$\chi^2 = 17.93$$

on similar crosses have shown that a one factor difference explains most of the results satisfactorily. However, the data in Table X show clearly that a single factor difference does not very well explain segregation for awns following a cross of Garnet and Kota. The method of classification used was not, in the opinion of the author, entirely satisfactory. The deficiency in class 3 and the excess in class 5 cannot be explained on the basis of errors in classification. It must consequently be concluded that probably more than one factor is involved in awn segregation in Kota \times Garnet crosses.

Strength of Straw

The ability to resist lodging and stand erect is a very important agronomic character in a variety of wheat. Straw that will resist lodging when conditions are unfavorable is sought in all hybrid selection work which aims at the improvement of the present varieties of wheat. However, strength of straw is a character that varies so greatly with differences in environment that few genetic studies have been made on it and no factorial explanation of its inheritance attempted.

The Howards (28) made a study of the standing power of wheats which they believed to be due to strong straw and the power to form a strong root system. They made crosses between a *vulgate* form with strong straw but inferior rooting capacity and another variety with weak straw but good rooting power. In F_2 all combinations of these characters appeared. This would seem to indicate independent inheritance of the two characters considered by the Howards to produce good standing power.

Harrington (20) made a study of erectness of plant in the parents and hybrids of two durum crosses. Grown in two series of rows the F_4 families of Mindum \times Pentad gave a correlation of $+0.389 \pm 0.037$ and of Kubanka No. 8 \times Pentad a correlation of $+0.537 \pm 0.041$. Transgressive segregation occurred in both crosses. He concluded "that in both crosses erectness is dependent on several heritable factors, part of which are present in one parent and part in the other". A slight linkage between factors governing rust resistance under field conditions and those governing erectness of plant was noted.

Waldron (52) points out that an attempt was made to secure a strong-strawed rust-resistant line from Kota \times Marquis crosses. Marquis has a strong straw while Kota is rather weak. Differences in strength of straw were very apparent in the selections made, the range being from about the strength of Kota to midway between the two parents. No rust-resistant line with strength of straw equal to Marquis was found. However, this author believes the occurrence of such a form to be possible. One resistant selection showed considerably weaker straw than the Kota parent.

An association of awnedness and increase in lodging in Propo hybrids grown at Davis, California, was noted by Clark, *et al.* (8). Goulden and Neatby (15) have reported that a genetic linkage appears to exist between weakness of straw and mature plant resistance to rust. This linkage, it is pointed out, may be broken if a large enough hybrid population is grown to permit the detection of the few cross-over types.

An attempt was made to classify the strength of straw or standing power in each row of the three F_3 series for all four populations. The parental check rows were first studied and the standing power of the parents taken as the basis of classification. For the purposes in view, upstanding rows of Red Bobs and Garnet were considered as strong and designated as "S". In each of these varieties some rows, affected by the fluctuations of environment, appeared less upstanding than the strong rows. These were classed as midstrong and designated as "MS". The midrange of the Kota straw strength was taken as representative of the weak, or "W" class. Kota showed a considerable tendency toward the production of knee-bent culms that spread outward from the upright plane of the row, and all culms showed a tendency to criss-cross within the row or to fall into or against adjacent rows. An intermediate class was made between the midstrong class and the weak, into which many of the stronger-appearing Kota parent lines fell, and a weaker class was set up to include those lines which showed distinct signs of lodging. These were known as midweak, and very weak, and designated "MW" and "VW" respectively.

Determinations were made following a period of wind and rain. At this time the earlier lines were in the dough stage but still green, and the later lines contained large watery kernels. About one week later these determinations were checked. No attempt was made to distinguish heterozygosity, the standing power of the whole row being taken into consideration. A final rating was made, where the rating in the replicates was at variance, by (1) taking the midclass if three contiguous ratings had been made, (2) by taking the majority rating where two were in agreement, and (3) by making adjustments in favor of the class rating represented by the largest number of plants where the lines were badly reduced in number by the epidemic of cutworms and the wind damage already referred to.

Examination of the data in Table XI reveals a complex condition. A leaning toward greater weakness than that found in Kota is evident in both crosses. If transgressive segregation were not taking place the expected

TABLE XI

DISTRIBUTION FOR STRENGTH OF STRAW IN PARENTS AND F_2 HYBRID LINES FROM
KOTA \times RED BOBS AND KOTA \times GARNET CROSSES

Parents and crosses	Numbers in classes for straw strength					
	S	MS	MW	W	VW	Totals
Kota	—	3	50	21	3	77
Red Bobs	33	11	—	—	—	44
Garnet	19	15	2	—	—	36
Kota \times Red Bobs	15	64	114	148	52	393
Kota \times Garnet	34	74	78	120	35	338

S = Strong; MS = Midstrong; MW = Midweak; W = Weak; VW = Very weak.

numbers of hybrid lines in the "VW" class would be of the order of 11 and 8 instead of 52 and 35 respectively.

The data were fitted to a theoretical single factor ratio. To do this the "S" and "MS" classes were grouped as "Strong" while the "MW" and "W", and "VW" classes were grouped as "Weak". Odds against the occurrence, due to chance, of deviations as great or greater than the ones obtained were 39 : 1 and 216 : 1 in the Kota \times Red Bobs and Kota \times Garnet crosses respectively. This indicates that the segregation cannot be explained on a single-factor basis.

In attempting to fit the data to a two-factor ratio it was assumed that the factors for strength of straw, designated S and S', were cumulative in effect and partially dominant. Since no attempt had been made to distinguish the heterozygous from the homozygous lines when taking the data it was expected that the classes set up on the basis of phenotype would contain progeny of plants differing in genotype in F_2 . The grouped weak and very weak classes would be derived from the *sss's'*, *ssS's'*, *Sss's'*, *SSs's'* and *ssS'S'* genotypes, the midweak class from the *SsS's'* genotype, the midstrong class from the *SSS's'* and *SsS'S'* genotypes and the strong class from the homozygous dominant, *SSS'S'*. The four classes so derived would be in the ratio of 7 : 4 : 4 : 1 respectively. Calculations showing the goodness of fit to this

TABLE XII

STRENGTH OF STRAW SEGREGATION IN KOTA \times RED BOBS F_2 HYBRID LINES AND
CALCULATIONS OF GOODNESS OF FIT TO A TWO-FACTOR RATIO

Strength class	Expected ratio	Numbers		O - C	(O - C) ²	$\frac{(O - C)^2}{C}$
		Observed	Calculated			
VW + W	7	200	171.9	28.1	789.6	4.59
MW	4	114	98.3	15.7	246.5	2.51
MS	4	64	98.3	34.3	1176.5	11.97
S	1	15	24.6	9.6	92.2	3.75

$$P = 0.000045$$

$$X^2 = 22.82$$

TABLE XIII

STRENGTH OF STRAW SEGREGATION IN KOTA \times GARNET F_2 HYBRID LINES AND
CALCULATION OF GOODNESS TO FIT TO A TWO-FACTOR RATIO

Strength class	Expected ratio	Numbers		O - C	(O - C) ²	$\frac{(O - C)^2}{C}$
		Observed	Calculated			
VW + W	7	155	149.2	5.8	33.6	0.23
MW	4	78	85.3	7.3	53.3	0.62
MS	4	74	85.3	11.3	127.7	1.50
S	1	34	21.3	12.7	161.3	7.57

 $P = 0.0194$ $X^2 = 9.92$

phenotypic ratio are given in Tables XII and XIII. In this case also the expected ratio was not close enough to the obtained results to warrant the conclusion that segregation for this character was entirely on a two-factor basis.

From a consideration of these attempts to fit the segregation for straw strength to single factor and two-factor ratios it would appear that proof for either explanation could not be offered. But if the difficulties of classification are taken into consideration a two-factor difference might well be indicated by the Kota \times Garnet data. No attempt was made to test for goodness of fit to a three-factor ratio, since it was believed that the classification was not fine enough; nor could it be made with sufficient accuracy on a character that is so easily modified by environment. It was concluded that the complex segregation noted in this study of strength of straw could be explained best on a multiple factor basis, the presence of two main factors being indicated.

Correlation Studies

The importance of linkage studies in plant breeding cannot be too strongly stressed. In connection with studies on the inheritance of disease reaction the use of correlation as a tool to uncover linkage relations is doubly important. A specific test must, as a rule, be made to determine the presence of factors for resistance and susceptibility. This would be less necessary if such factors were linked with some easily distinguishable morphological character. Under varying environmental conditions it is not always possible to obtain an adequate disease reaction test. Thus the process of selecting desirable, disease resistant agronomic forms from a hybrid population is made difficult. Linkage relations when found are seldom of a complete nature, *i.e.*, a certain amount of crossing-over occurs. A knowledge of the strength of linkage is therefore important in estimating the chances of obtaining a suitable recombination where linked desirable and undesirable characters enter a cross.

That qualitative characters may be linked with quantitative and other characters has been amply demonstrated. Lindstrom (32) found that the number of rows in the ear of corn (*Zea mays*), a quantitative character, was

associated in inheritance with cob, aleurone, and endosperm color as well as with endosperm texture, all of which are simple qualitative characters. Griffiee (18), working with barley (*Hordeum* spp.) demonstrated very striking linkage relations between morphological characters and reaction to *Helminthosporium sativum*. Three factors were found to be concerned with the production of resistance of a certain type. One of these factors was found to be linked with the factor for two-rowed, one with the factor for rough awn, and another with the factor for white glumes. He reports the linkage of the factor for early heading, a physiological character, with the factor for six-rowed and the factor for susceptibility to *Helminthosporium sativum*.

In the studies here reported an investigation was made of the relation between the reaction of Kota bunt and Red Bobs bunt, and between bunt reaction and some plant characters in the F_3 of Kota \times Red Bobs and Kota \times Garnet crosses. Since the data on bunt reaction were taken on a uniform class basis it was possible to use the correlation coefficient (Pearson's " r ") to measure the degree of correlation between the reactions of the two bunts. In the case of the plant characters the classes used could not be considered to have any specific numerical relation. This necessitated the use of the correlation ratio, η . A comparison of the values of r and η in some distributions by means of Blakesman's test for linearity showed that the correlation ratio was the better measure of correlation for those cases. The probable errors were computed by standard formulas. Although the correlation ratio η does not normally carry a plus or minus sign these were added to indicate whether the general trend of correlation was found to be positive or negative (5, 26, 41).

Kota \times Red Bobs hybrids inoculated with Kota bunt. The two characters, awning and strength of straw were correlated with the reaction of the F_3 hybrid plants to bunt.

Negative correlation ratios between reaction to Kota bunt and awning, and Kota bunt and straw strength were obtained. These were -0.262 ± 0.037 and -0.303 ± 0.036 respectively. This would seem to indicate that the Kota plant character, weak straw, is associated with susceptibility to Kota bunt. Some of the genetic factors governing the expression of this character must be linked with a factor for bunt reaction, and therefore located on the same chromosome. Factors for the Kota plant character, awnedness, would seem to be linked with factors for resistance to Kota bunt.

Kota \times Red Bobs hybrids inoculated with Red Bobs bunt. The correlation of the same two characters with reaction to Red Bobs bunt in the F_3 lines of these crosses was positive. The correlation ratio for reaction to Red Bobs bunt and awning was $+0.238 \pm 0.052$, and for reaction to Red Bobs bunt and strength of straw, $+0.204 \pm 0.051$. This would seem to indicate that the Red Bobs character of stronger straw was associated with susceptibility to Red Bobs bunt. Some of the genetic factors governing the expression of this character must be linked with some factor for bunt reaction and therefore located on the same chromosome. In the case of the awnless character

of Red Bobs the significant positive correlation indicates a linkage between awnless factors and resistance to the Red Bobs bunt.

Kota × Garnet hybrids inoculated with Kota bunt. The correlation value of -0.203 ± 0.041 between reaction to Kota bunt and strength of straw indicates that the linkage between factors for susceptibility to Kota bunt and for weak straw, noted in the Kota × Red Bobs crosses, has also been carried into this cross.

The negative correlation ratio of -0.235 ± 0.027 obtained for reaction to Kota bunt and awning also parallels the findings in regard to these characters in the Kota × Red Bobs crosses.

Kota × Garnet hybrids inoculated with Red Bobs bunt. Reaction of Red Bobs bunt correlated with strength of straw in these crosses gave a significant correlation ratio of $+0.191 \pm 0.043$, indicating that as strength of straw increases the susceptibility to Red Bobs bunt also increases. This parallels the results for the Kota × Red Bobs crosses and would indicate that some of the same factors for strength of straw are probably present in both Garnet and Red Bobs.

The reaction to Red Bobs bunt and awning show a correlation ratio of $+0.274 \pm 0.041$. That the relation would be of a positive nature is to be expected if the resistance factor of Garnet is linked with factors for short tip awn.

TABLE XIV

SUMMARY OF CORRELATION RATIOS AND CORRELATION COEFFICIENTS OBTAINED IN STUDIES OF CORRELATED INHERITANCE IN F_2 HYBRIDS OF KOTA × RED BOBS AND KOTA × GARNET CROSSES

Characters and reactions correlated	F_2 hybrids	Correlation coefficient
Kota bunt and Red Bobs bunt	Kota × Garnet	$+0.373 \pm 0.042$
Kota bunt and Red Bobs bunt	Kota × Red Bobs	$+0.082 \pm 0.059$
		Correlation ratios
Kota bunt and strength of straw	Kota × Red Bobs	-0.303 ± 0.036
Kota bunt and awning	Kota × Red Bobs	-0.262 ± 0.037
Red Bobs bunt and strength of straw	Kota × Red Bobs	$+0.204 \pm 0.051$
Red Bobs bunt and awning	Kota × Red Bobs	$+0.238 \pm 0.052$
Kota bunt and strength of straw	Kota × Garnet	-0.203 ± 0.041
Kota bunt and awning	Kota × Garnet	-0.235 ± 0.027
Red Bobs bunt and strength of straw	Kota × Garnet	$+0.191 \pm 0.043$
Red Bobs bunt and awning	Kota × Garnet	$+0.274 \pm 0.041$

Summary of correlation values. In Table XIV a summary is given of the correlation values derived in the course of the inheritance studies just reported. With the exception of one value, they may be considered significant. They

are, with the exception noted, of the same order and, while not large, may be considered highly significant for the purposes of the present study. Attention is drawn to the use by Harris (23), in his study of biological reactions in cotton, of similar small but statistically significant correlation coefficients.

Kota × Red Bobs hybrids. The correlation between the reactions of Kota × Red Bobs F_3 lines inoculated with bunt from Kota and from Red Bobs was $+0.082 \pm 0.059$. This non-significant value was expected in view of the susceptibility of both parents to both smuts. The wide scatter on this correlation surface suggested, however, that not all of the susceptibility found in the F_3 lines of these crosses was due to the same factors.

Kota × Garnet hybrids. In the Kota × Garnet F_3 lines the correlation between the reactions obtained by inoculation with the two original bunt collections is indicated by a coefficient of $+0.373 \pm 0.043$. Garnet is resistant and consequently there was a differential reaction among the various hybrid lines. The significant positive correlation indicates that the factor (or factors) for resistance in Garnet governs the reaction to both bunts.

As in the case of the Kota × Red Bobs lines further tests would have been necessary to prove the differential nature of certain F_3 lines that were very susceptible to Kota bunt and resistant to bunt from Red Bobs. It should be noted that no lines resistant to Kota bunt yet highly susceptible to Red Bobs bunt were found, although some lines were resistant to both kinds. The F_4 hybrid lines that were tested in 1931 came from a group comprised approximately of one-quarter of the total number of hybrid F_3 lines on which the double test had been made. They had shown in 1930 from 0 to 10% infection from Red Bobs bunt and 0 to 25% infection from Kota bunt and they continued to show some resistance in F_4 . A few lines showing considerable resistance will be tested further. As in the F_3 a significant positive correlation was found between the reactions of the two bunt collections used on the hybrids. In this case $r = +0.458 \pm 0.042$.

These findings bear out the earlier conclusions that Garnet resistance was operative against both bunts and would indicate that such resistance is conditioned by a single genetic factor.

Summary

The studies herein reported were made on the F_2 , F_3 and part of the F_4 population, in crosses between Kota and Red Bobs, and between Kota and Garnet. Duplicate populations in each cross proved to be similar in nature as shown by similarity in reaction to bunt and loose smut, by seed color segregation and by other characters studied.

Studies were made of inheritance of resistance to bunt from Kota and bunt from Red Bobs, of resistance to Kota loose smut, and of the characters, seed color, awns, and straw strength. Correlated inheritance studies were

also made of the reaction of the hybrids of the two crosses to the two bunt forms, and of the reaction of Kota bunt and Red Bobs bunt to awning and strength of straw.

Kota and Red Bobs were highly susceptible to both Kota bunt and Red Bobs bunt. Garnet was resistant to both bunts but less resistant to the Kota bunt.

The original bunt collections were considered to be essentially different in regard to physiologic reaction as well as in morphological character. The Kota bunt (*Tilletia levis*) was much more virulent than the Red Bobs bunt (mainly *T. tritici*).

The heritable nature of bunt reaction was clearly demonstrated. The percentage of infection found in the parent lines was recovered in the F_3 hybrids but no transgressive segregation was noted. An indication of the dominance of the bunt-resistance factors was obtained and the presence of several factors governing bunt reaction indicated.

Kota was relatively susceptible to loose smut, Garnet resistant, and Red Bobs immune under the conditions of test. The heritable nature of the reaction to loose smut apparently indicated by the close check in percentage of infection in the duplicate populations and by the relative percentages of infected lines from the F_3 of susceptible \times resistant and susceptible \times immune crosses, was not evident in the F_4 tests. Infection of the hybrid Kota \times Garnet population appeared to take place entirely in a random manner. It is suggested that such resistance as was apparent in the parental varieties might have other than a physiological basis.

Two factor pairs are believed to be concerned with segregation for color in the Kota \times Red Bobs hybrids. Since both parents are red it must be concluded that each carries a single factor pair for red color, unlike that carried by the other. Failure to obtain a good fit for the segregating classes in F_3 was not entirely explainable. Taken in conjunction with other findings in regard to seed coat color this study points to the need for a thorough investigation of this character with the practical end in view of obtaining homogeneity for color in our red wheat varieties.

The segregation observed for awn characters in the F_3 lines agreed with the results of Clark (6, 8, 9) from similar crosses. His method of awn classification (6) was used in preference to that of Stewart, but it was not found entirely satisfactory. It was concluded that more than one factor was probably involved in awn segregation in the Kota \times Garnet hybrids and at least two in the Kota \times Red Bobs hybrids.

Indications were obtained that a two-factor difference conditioned the segregation in strength of straw in the crosses of Kota \times Garnet. However, the complex segregation noted in this study of straw strength can best be explained on a multiple-factor basis. Transgressive segregation in the direction of greater weakness of straw was noted.

Correlation studies between the reactions of Kota \times Garnet hybrids to bunt from Red Bobs and Kota indicated that the same factor for resistance in Garnet governs the reaction to both bunts. Garnet resistance was found to be conditioned by a single partially dominant factor pair.

Considering the reaction of the two bunts used to inoculate the Kota \times Red Bobs hybrids it is noteworthy that, in both cases, susceptibility to bunt from a parent is associated with the strength of straw of that parent. Also in both cases the degree of resistance to the bunt from the parent is associated with the characteristic awn type of that parent. The conclusions to be drawn from the reactions of the two bunts with the Kota \times Garnet hybrids bear out the findings in regard to the Kota \times Red Bobs hybrids. Linkage between factors for strength of straw and susceptibility to bunt and a similar linkage between awn type factors and bunt resistance factors are evident.

Taken together the last-mentioned findings demonstrate the presence in wheat of two linkage groups concerned with the reaction to bunt forms. However, since the expression of both strength of straw and awn type appears to be conditioned by two or several factors it is believed that the linkage may involve only one pair concerned with each character. The correlation obtained, while significant, indicates that linkage is not complete in either case, nor can it be considered strong enough to interfere greatly with breeding for bunt resistance in wheat.

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PYOCYANINE AND GROWTH POTENTIAL CHANGES OF *PS. PYOCYANEUS*¹

BY G. B. REED² AND E. M. BOYD³

Abstract

The pigment pyocyanine, produced by *Ps. pyocyaneus*, together with its leuco form has been shown by Friedheim and Michaelis to constitute a reversible oxidation-reduction system. Friedheim has shown that under certain circumstances the pigment may act as a respiratory enzyme. In this paper it is shown that two ordinary types of culture media, when sterile and freely exposed to the air, exhibit potentials more positive than that of pyocyanine. With the growth of *Ps. pyocyaneus* the potential, in the depths of the fluids, falls to a more negative value than that of the leuco form of this pigment. The surface layer exposed to air remains more positive than the potential of pyocyanine. The surface layer of the cultures therefore becomes colored; the deeper layers remain colorless.

These changes in potential in the culture fluid make ideal conditions for the functioning of pyocyanine as a respiratory catalyst—at the surface of the medium it is oxidized, in the depths reduced. The observation that a non pyocyanine-producing strain gives approximately the same growth-oxidation-reduction curves apparently indicates that the potential changes are not dependent on the pigment.

It was recently shown by Friedheim and Michaelis (6) and Michaelis (11) that the blue pigment, pyocyanine, produced by *Ps. pyocyaneus*, in mixture with its leuco compound constitutes a reversible oxidation-reduction system which at pH 6.0 and more alkaline reactions behaves like any of the known reversible dyestuffs of quinoid structure. Later Friedheim (5) demonstrated that the consumption of oxygen by washed cells of *Ps. pyocyaneus*, by other species of bacteria and by rabbit red cells, is increased in the presence of pyocyanine; the pyocyanine may act therefore as an accessory respiratory enzyme. A study of the oxidation-reduction potentials of growing cultures of *Ps. pyocyaneus*, which was in progress at the time the above papers were published, gives further evidence of the biological significance of this substance.

The Eh of completely oxidized pyocyanine at pH 7.25, according to Friedheim and Michaelis, is approximately +0.080 volts; that of the reduced form, the leucopyocyanine, -0.100 volts, and that of a mixture of equal parts of oxidized and reduced pyocyanine, -0.047 volts. Elema's (4) demonstration that from pH 6 to 9 the Eh of the pyocyanine system lies between that of methylene blue and indigo trisulphonate indicates in a less precise fashion the same reaction. Since pyocyanine can function as a respiratory catalyst only in a medium capable of potential change through this Eh range, the question arises as to whether, in ordinary cultures of *Ps. pyocyaneus*, such conditions exist. A series of growth-potential determinations were therefore undertaken.

The technique was similar to that described in a former paper, Boyd and Reed (3). More uniform results were obtained by using greater care in selecting

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and cleaning the platinum electrodes and in discarding those which deviated by more than 20 millivolts from the standard when tested in a solution of known potential. The culture-electrode vessels consisted of 250-cc. Erlenmeyer flasks stoppered with ordinary cotton plugs through which was passed the tube supporting the platinum electrode and one arm of a potassium chloride-agar bridge. The flasks were almost filled with fluid media, 200 cc., the platinum electrodes put in place near the bottom, and the cotton stoppers arranged with a removable glass rod in the opening for the potassium chloride-agar bridge. They were then autoclaved and, after cooling, the sterile agar bridges put in place. The necks of the flasks were large enough to allow free circulation of air through the cotton plugs. Cultures were incubated and tested at 37° C.

I

Flasks of beef extract broth containing 0.1 *M* phosphate as a buffer and adjusted to pH 7.2 were arranged as half-cells in the manner just described. After autoclaving they were allowed to stand for 48 hr. until the major positive drift in potential, consequent on the heating, had passed, in order that more uniform initial potentials in the different flasks might be ensured, as previously discussed by Boyd and Reed (2).

The flasks were seeded with 0.1 cc. of a saline emulsion of organisms from a young agar culture. In each experiment three half-cells were inoculated and two similar cells without organisms were used as controls. The results of one such experiment are shown in Table I and the mean Eh values plotted in Fig. 1.

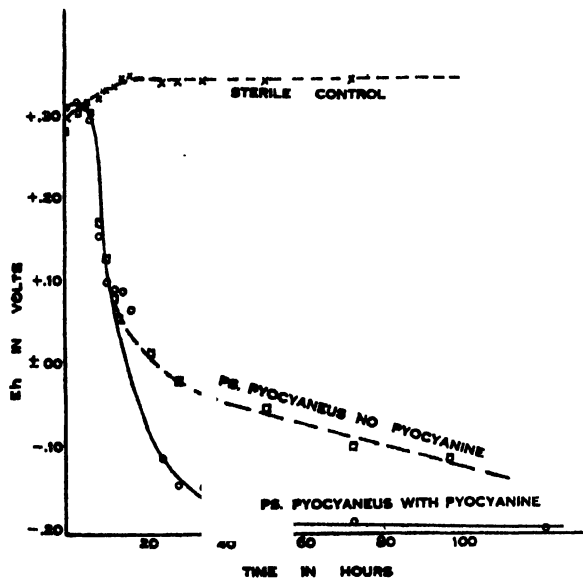


FIG. 1. Curves showing the oxidation-reduction potential of cultures of *Ps. pyocyaneus* growing in buffered broth: lower curve, a pyocyanine producing strain, data from Table I; middle curve, a non-pyocyanine producing strain, data from Table III; upper curve the sterile broth. Ordinates, Eh in volts; abscissas, time in hours.

Growth was perceptible as a faint clouding of the medium in about eight hours. At this point, as indicated in Fig. 2, a precipitous drop in the potential began and continued for eight to ten hours to an Eh of approximately -0.10 volts. This was followed over a period of three weeks by a much more gradual decline to some -0.234 volts and by a slight positive drift during the fourth week. The culture fluid was sufficiently buffered that there was no change in pH. During this period the sterile broth controls remained practically constant at an Eh of +0.30 to +0.35 volts. Several series of cultures gave similar results.

The persistence of this low potential in the cultures for so long a period appears to be unique for cultures freely exposed to atmospheric air. Under similar circumstances cultures of streptococci (7), *B. diphtheriae* (8), staphylococci (9), and pneumococci (10) maintain low reducing potentials for only a few hours.

Pyocyanine first appeared in these cultures after 20-24 hr. as a faint blue-green coloration of the surface layer of the broth. The color gradually deepened to an intense blue-green but the colored material remained restricted

TABLE I
GROWTH POTENTIALS OF *Ps. pyocyaneus* IN BUFFERED BROTH AT pH 7.2

Time, hr.	Sterile controls			Cultures of <i>Ps. pyocyaneus</i>			
	1	2	Mean	1	2	3	Mean
	Eh, volts						
0	0.300	0.302	0.301	0.314	0.318	0.311	0.314
3	0.307	0.322	0.315	0.320	0.318	0.322	0.320
5	0.310	0.325	0.318	0.319	0.315	0.315	0.317
6	0.312	0.326	0.319	0.289	0.290	0.288	0.289
8	0.322	0.327	0.325	0.165	0.154	0.150	0.156
10	0.333	0.339	0.336	0.110	0.102	0.107	0.106
12	0.336	0.345	0.341	0.090	0.095	0.104	0.096
14	0.346	0.354	0.350	0.090	0.094	0.098	0.094
16	0.350	0.352	0.351	0.079	0.071	0.059	0.070
24	0.345	0.342	0.344	-0.100	-0.108	-0.123	-0.110
28	0.342	0.348	0.345	-0.132	-0.138	-0.152	-0.141
34	0.344	0.349	0.347	-0.140	-0.142	-0.150	-0.144
50	0.343	0.348	0.346	-0.178	-0.188	-0.188	-0.185
72	0.348	0.350	0.349	-0.187	-0.183	-0.188	-0.186
168	0.390	0.390	0.390	-0.192	-0.188	-0.208	-0.196
240	0.404	0.406	0.405	-0.204	-0.200	-0.225	-0.210
288	0.416	0.417	0.417	-0.221	-0.223	-0.234	-0.226
504	0.427	0.426	0.427	-0.223	-0.236	-0.245	-0.234
692	0.461	0.468	0.464	-0.188	-0.225	-0.166	-0.193

to the surface zone (1-2 cm. in thickness). This is in agreement with the results of the potential determinations. By the time enough pyocyanine had formed to perceptibly color the fluid, the potential in the depths, as determined by the electrodes placed near the bottom of the flasks, had fallen to an Eh of less than -0.10 which, according to Friedheim and Michaelis (6), is the potential of the leuco form of the pigment.

II

Ps. pyocyaneus grows luxuriantly on certain synthetic culture media, particularly the asparagin-glycerol-salt medium of Proskauer and Beck, commonly used for tubercle bacilli. To prevent changes in pH during growth, Proskauer and Beck's medium as modified by Kock (Baldwin, Petroff and Gardner (1)) was further modified by increasing the concentration of disodium hydrogen phosphate to ten grams per litre and adjusting the pH

with sodium hydroxide to 7.2.* Flasks containing this medium were arranged as half-cells, exactly as with broth, described in the previous section, inoculated from a young agar culture and the potential determined as in the previous instances. The results of one experiment are indicated in Table II and Fig. 2.

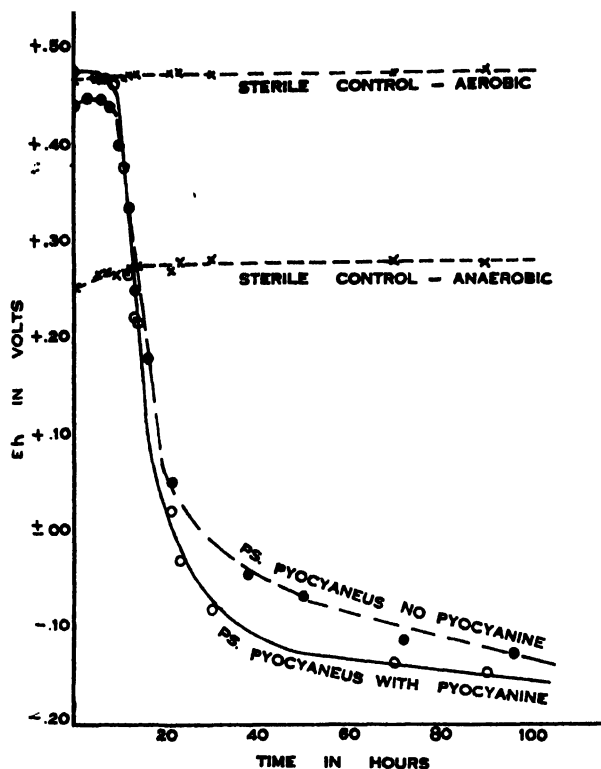


FIG. 2. Curves showing the oxidation-reduction potential of cultures of *Ps. pyocyaneus* growing in Proskauer and Beck's synthetic media: lower curve, a pyocyanine producing strain, data from Table II; second curve, a non-pyocyanine producing strain, data from Table III; two upper curves, the sterile medium under aerobic and anaerobic conditions. Ordinates, E_h in volts; abscissas, time in hours.

tial in the cultures, indicated in Table II and Fig. 2, must result directly from the metabolism of the bacteria.

Change in potential in the depths of cultures in this synthetic medium was similar to that in broth cultures. The precipitous fall began, as indicated in Fig. 2, nine or ten hours after the culture was inoculated and growth became perceptible as a faint clouding of the medium about two hours later. Both the conspicuous potential change and the gross indication of growth

With this medium two sets of sterile controls were used; one freely exposed to the air through cotton stoppers and one set with a vaseline seal over the surface of the fluid. It will be observed that in both instances the potential remained high and practically unchanged during the period of the experiment. This is in marked contrast with the behavior of sterile broth. Several observers have reported a fall in the potential of sterile broth under anaerobic conditions. In a former paper (2) evidence was presented which indicated that this results from the absorption of oxygen or other oxidizing bodies by the broth. It seemed possible that the fall in potential in the depths of growing cultures of *Ps. pyocyaneus*, and other bacteria, might result from similar changes. Since the aerobic and anaerobic sterile controls in this synthetic medium remain unchanged during the period of the experiment, it seems evident that the fall in poten-

*At this pH it is necessary to sterilize the medium in two parts in order to prevent the precipitation of magnesium phosphates. The magnesium sulphate and half the water is placed in one vessel, the other ingredients and the balance of the water in a second vessel. After autoclaving and cooling the two are combined.

TABLE II

GROWTH POTENTIALS OF *Ps. pyocyaneus* IN BUFFERED SYNTHETIC MEDIUM AT pH 7.0

Time, hr.	Sterile controls						Pyocyanus, aerobic		
	Aerobic			Anaerobic					
	1	2	Mean	1	2	Mean	1	2	Mean
	Eh volts								
0	0.470	0.476	0.473	0.250	0.262	0.256	0.484	0.477	0.481
5	0.471	0.476	0.474	0.262	0.278	0.270	0.481	0.471	0.476
7	0.474	0.474	0.474	0.264	0.279	0.272	0.476	0.267	0.472
9	0.473	0.477	0.475	0.264	0.274	0.269	0.471	0.462	0.467
11	0.476	0.478	0.477	0.268	0.280	0.274	0.385	0.380	0.383
12	0.479	0.478	0.478	0.270	0.286	0.278	0.266	0.277	0.272
13	0.479	0.478	0.478	0.268	0.287	0.278	0.215	0.237	0.226
14	0.478	0.480	0.479	0.271	0.285	0.278	0.204	0.233	0.219
21	0.480	0.480	0.480	0.276	0.271	0.274	0.026	0.022	0.024
23	0.480	0.479	0.480	0.285	0.276	0.281	-0.036	-0.023	-0.030
30	0.478	0.478	0.478	0.292	0.281	0.287	-0.086	-0.075	-0.081
70	0.478	0.480	0.479	0.281	0.288	0.285	-0.133	-0.138	-0.136
90	0.477	0.483	0.480	0.272	0.291	0.282	-0.144	-0.151	-0.148
120	0.476	0.482	0.479	0.260	0.282	0.271	-0.137	-0.134	-0.136
168	0.475	0.482	0.479	0.264	0.276	0.270	-0.123	-0.134	-0.129
240	0.478	0.483	0.481	0.267	0.274	0.271	-0.141	-0.145	-0.143
288	0.478	0.484	0.481	0.270	0.280	0.275	-0.142	-0.147	-0.145
504	0.480	0.486	0.483	0.285	0.278	0.282	-0.143	-0.152	-0.148
692	0.484	0.488	0.486	0.260	0.284	0.272	-0.157	-0.155	-0.156

were therefore three to four hours later than in broth. The subsequent potential changes, it may be observed, were essentially the same as in the broth cultures although the maximum reducing potential in the synthetic medium was slightly less than in the broth. It is significant however from the point of view of the pyocyanine that an Eh of -0.10 volts was reached after 30-40 hr. growth and remained below this level for the period of the experiment, some four weeks, *i.e.*, below the level of oxidized or blue pyocyanine.

The blue pigment was apparent after 18-20 hr. growth, as in the case of the broth cultures, in a surface zone never more than a centimetre in thickness. The pigmentation gradually became more intense but remained restricted to the surface notwithstanding a heavy diffuse growth throughout the medium.

III

In the course of a study of the variation of *Ps. pyocyaneus* Reed (12) isolated a strain which produced no pyocyanine, though in other respects it was typical of the species. This strain was grown in broth and in the synthetic medium and the potential changes followed under the same conditions as described in two previous sections. The results of one such experiment are indicated in Table III and plotted in Figs. 1 and 2. It is evident that in both media the non pyocyanine-producing variant produced essentially the same

growth-potential curves as the typical pyocyanine-producing strains, although the final potential with the former was slightly less negative than in pigment-forming cultures. Cultures of both strains however assumed an Eh *more negative* than the Eh of leucopyocyanine.

It seems evident then that the oxidation-reducing potentials in cultures of this organism are not primarily due to pyocyanine.

IV

It is evident from these experiments that the two types of culture media, when sterile and freely exposed to air, give oxidation potentials which are more positive than the Eh of pyocyanine and, when under anaerobic conditions, the synthetic medium remains unchanged while broth gradually assumes a potential more negative than that of leucopyocyanine. Cultures of *Ps. pyocyaneus* in both media develop, in the depths, a potential more negative than that of leucopyocyanine, while the surface layer exposed to the air remains more positive than pyocyanine. As a result, in the case of

TABLE III

GROWTH POTENTIALS OF A NON-GREEN PYOCYANINE FORMING STRAIN OF *Ps. pyocyaneus* IN BUFFERED BROTH AND PROSKAUER AND BECK'S MEDIUM

Time, hr.	Growth potentials in buffered broth			Growth potentials in Proskauer and Beck's medium		
	1	2	Mean	1	2	Mean
	Eh volts					
0	0.276	0.295	0.286	0.422	0.474	0.448
3	0.309	0.311	0.310	0.430	0.489	0.460
5	0.316	0.319	0.318	0.430	0.488	0.459
6	0.311	0.307	0.309	0.431	0.482	0.457
8	0.177	0.177	0.177	0.430	0.459	0.445
10	0.143	0.121	0.132	0.406	0.401	0.404
12	0.072	0.084	0.078	0.380	0.301	0.340
13	0.049	0.070	0.060	0.257	0.245	0.251
16	0.083	0.087	0.085	0.184	0.182	0.183
21	0.017	0.016	0.017	0.024	0.082	0.053
28	-0.011	-0.016	-0.014	-0.033	-0.053	-0.043
50	-0.032	-0.066	-0.049	-0.055	-0.085	-0.065
72	-0.076	-0.092	-0.084	-0.096	-0.123	-0.110
96	-0.111	-0.113	-0.112	-0.112	-0.136	-0.124
168	-0.138	-0.154	-0.146	-0.104	-0.105	-0.105
336	-0.191	-0.193	-0.192	-0.121	-0.112	-0.117
504	-0.206	-0.204	-0.205	-0.092	-0.104	-0.098
672	-0.211	-0.212	-0.212	-0.073	-0.118	-0.096

the ordinary pyocyanine-producing strains, the surface layers become blue while the deeper portion of the fluid remains colorless. With diffusion therefore the pyocyanine will be alternately oxidized and reduced. This appears to constitute ideal conditions for the functioning of the pigment as a respiratory catalyst.

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THE CHEMICAL AND PHYSIOLOGICAL PROPERTIES OF CRYSTALLINE OESTROGENIC HORMONES¹

By J. S. L. BROWNE²

Abstract

A crystalline oestrogenic hormone has been isolated from human placenta. This substance shows a melting point of 274° C. (uncorrected). The carbon-hydrogen analysis gives: carbon, 74.8%; hydrogen, 8.8%. The mixed melting point with theelol shows no lowering. The substance differs physiologically from previously isolated female sex hormones. It is relatively inactive on the adult ovariectomized albino rat. The dose required is 16 γ as compared with 1.5 γ for theelol. It is, however, effective on the immature intact rat, 21 days old, in a dose of 1.2 γ . This is the same as theelol. On the immature castrate, the dose of the placental crystals is 10 γ , that for theelol is 3 γ . The presence of the ovary is believed to be the factor in the dosage ratio between adult spayed and normal intact animals. Transplantation of immature ovaries into immature or adult castrates, even though it does not render them cyclic, causes the weight of the placental crystals needed for an effect to fall to the immature intact dose.

The isolation of a crystalline substance having oestrogenic properties was first reported by Doisy and coworkers in 1929 (22). Shortly after Butenandt also described the preparation of such a crystalline material (8). Marrian in 1930 reported the isolation of a crystalline substance having oestrogenic properties which he believed to differ from those of previously isolated crystals (24, 25). It has been shown by the work of Butenandt (9) and of Doisy and coworkers (34) that the first isolated substance called by Doisy "theelin" has an empirical formula of $C_{18}H_{22}O_2$ and contains one ketone and one hydroxyl group. The substance first isolated by Marrian (24, 25) and later by Butenandt (9, 10) and by Doisy and coworkers (20, 21, 33) has been shown to have an empirical formula of $C_{18}H_{24}O_3$, *i.e.*, to contain the elements of one more molecule of water than the keto-hydroxy form. It has three hydroxyl and no ketone groups. It has been called "theelol" by Doisy, and Marrian (26) uses the term "tri-hydroxy oestrin" to designate it. Butenandt (9, 10) has been able by heating the trihydroxy form in high vacuum with potassium acid sulphate to convert it to the keto-hydroxy form. In 1931 Marrian (26) on physiological grounds which will be discussed later suggested that two forms of keto-hydroxy oestrin existed. These he called "A" and "B" forms. He believed that the product obtained by Butenandt who used high vacuum distillation in his method was the "A" form and that Doisy's theelin was the "B" form. Butenandt and Störmer (11) in a paper just received have brought forward further evidence on both chemical and physiological grounds that two such isomeric forms exist. Their alpha form melts at 255° C. (uncorrected) and has an optical rotation in chloroform of $[\alpha]_D = 156-158^\circ$ and its benzoate shows a constant melting point at 217.5° C. (uncorrected). The β -hormone melts at 257° C. (uncorrected) has a rotation

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of $[\alpha]_D = 165-166^\circ$ and its benzoate shows two polymorphic forms, one melting at 220.5° C. and the other at 205° C. (both uncorrected). The greatest difference as discussed below lies in their physiological effect. Both forms are obtained on distilling the trihydroxy form in high vacuum, a slow distillation at $130-140^\circ$ C. and 0.02 mm Hg. tending to give the α -form and a rapid distillation at 200° C. tending to give the β -form. It is uncertain whether these isomers represent the "A" and "B" forms of Marrian (26) or not. Concerning the further structure of the oestrogenic substance Bernal (7) published evidence based on X-ray crystallography and Adam and coworkers (1) evidence based on surface films as an index of molecular dimensions tending to show that the crystalline oestrogenic substances possessed a structure like that of phenanthrene derivatives. Butenandt, Störmer and Westphal (12) in a very recent paper have reported extensive chemical investigations which lead to the conclusion that the phenanthrene derivative type of formula is the correct one. They offer tentative partial structural formulas and account for the presence of isomeric forms in the structure of the molecule. These formulas have been revised still more recently, and a four-ringed structure is now accepted.

Physiological Effect

The assay of these crystalline substances as to their oestrogenic effects has been performed almost wholly using the vaginal smear method on the ovariectomized adult white rat or mouse. There are many variable factors which affect the accuracy of this method of assay (2, 3, 6, 13, 23, 28,29). The condition of the animal, as affected by age, nutrition, the presence of infection and the length of time since ovariectomy, are important in influencing the result obtained. Variation in individual susceptibility in animals has been emphasized by Coward and Burn (13) and others (2, 4, 5) and the necessity for use of large numbers of animals has been recognized. Variations in the method of administration, such as injecting in oil or water and subcutaneously or otherwise, play a role in governing the response. Butenandt (11) reports that when the dose is divided into four or six injections the injection medium has little effect on the result. Perhaps the most important assay factors are the degree of division of the total dose into separate fractions, the time interval between the injections and the length of time between assays. There is no general agreement as to the criterion of a positive response, some authors requiring a full squamous flush and some only a disappearance of leucocytes and the presence of large numbers of nucleated epithelial cells. The number of animals which must show such a positive response, before the amount injected is considered a unit, may be set at 50 or 75% or some other fraction of the total number in the test. The discovery of two forms of keto-hydroxy oestrin and Butenandt's finding that the trihydroxy form tends to form mixed crystals with the keto-form makes uncertain the variations in potency which may occur in different laboratories. As a result of differences in composition of the crystalline material all of these variations make it difficult to compare the values obtained for a unit in

different laboratories. Table I shows the weight of material in terms of γ (thousandths of a milligram) required for a unit by various authors for the different crystalline preparations.

The conditions of assay are given in summary. In this table the influence of division of the dose into several injections in Butenandt's assays on α follicular hormone is very obvious.

If the substance is given in four injections it requires only about one-fifth the dose for the same response as when given in a single injection. The difference in sensitivity between the rat and the mouse is difficult to deter-

TABLE I
ASSAY OF CRYSTALLINE OESTROGENIC SUBSTANCES

Form	Compound and author	Potency γ per unit	Animal	No. of injections	Time interval	No. of animals	Per- centage response per unit
Keto- hydroxy	Theelin (Dolsy) (21)	0.02 0.38 1.08 1.08	A.M. A.R. I.R. I.R.	4 (W) 3 (W) 3 (W) oral (W)	36 12 48 7	— 20 5 5	— 75 60 60
	Follicular hormone Butenandt (9, 10, 11) α -Form	0.025 0.03 0.07 0.125 0.100 0.125 0.80 2.00 0.167 Inactive	A.M. A.M. A.M. A.M. A.M. A.M. A.M. A.M. A.M.	6 (O) 4 (O) 3 (O) 1 (O) 1 (O) 6 (O) 1 (O) 1 (O) 1 (O)	48 36 12 — — 48 — — —	10 10 12 12 10 10 10 — 12	80 80 80 80 80 80 80 — 80
	Becker <i>et al.</i> (6)	0.38	A.M.	1 (O)	—	100	50
	Gustavson and d'Amour (4, 5)	0.38	A.R.	1 (O)	—	100	50
	Marrian (26)	0.025	A.M.	4 (W)	36	20	50
	Keto-hydroxy A	0.060	A.M.	4 (W)	36	20	50
	Keto-hydroxy B						
Tri- hydroxy	Marrian (25, 26)	0.13	A.M.	4 (W)	36	20	50
	Butenandt (10, 11) Hydrate, m.p., 279° C.	13.3	A.M.	1 (O)	—	8	90
	272-273	10.0	A.M.	1 (O)	—	9	78
	266-268	4.0	A.M.	1 (O)	—	7	86
	263	2.5	A.M.	1 (O)	—	8	88
	Theelol (Dolsy) (19, 22) (276° C.)	0.68 0.16 0.32 1.35-2.0	A.R. I.R. I.R. A.R.	3 (W) 3 (W) oral (W) oral (W)	12 48 — —	20 5 5 ?	75 60 — —
	Emmenin crystals (274° C.)	16.0 1.55	A.R. I.R.	6 (W) 6 (W)	36 36	100 30	60 47

NOTE:—A = adult, ovariectomized; I = immature (normal); R = rat; M = mouse; W = water medium; O = oil medium.

mine. According to Becker *et al.* (6) the dose for the rat and mouse is the same. According to most other authors the mouse requires only about one-fourth the dose required by the rat. Recently Thayer and MacCorquodale (35) reported that the mouse was about 12 times as sensitive as the rat for theelin but had about the same sensitivity as the rat to theelol. In connection with Doisy's unit of 0.68 γ for theelol in the adult rat Thayer and MacCorquodale, working in Doisy's laboratory, found that the unit of theelol varied enormously according to whether the full squamous or the epithelial smear was required for a positive reaction. They found, on the other hand, that in the case of theelin there was little difference between the two criteria. In other words, all the rats which showed an epithelial response in the case of theelin later progressed to the squamous stage, but in the case of theelol assays, many rats reached an epithelial stage and then showed regression to a negative smear without ever passing through the squamous stage. Because of this finding the amount of theelol for a unit is considered by Doisy to be much greater than 0.68 γ (actually about 6.00 γ) when a full squamous flush is required before an animal is considered to show a positive reaction. The values of 1.08 γ for theelin and 0.16 γ for theelol, obtained for a unit by Doisy with the immature rat, are based on the opening of the vagina within 10 days of a first injection given at 19 days of age.

As seen in the table and as mentioned above, Marrian reported in 1931 (26) that there are two forms of keto-hydroxy oestrin. He also believes that the first and more active form A is obtained when the substance is purified by high vacuum distillation according to Butenandt's method. He believes that the A form changes into the B or less active form on standing even in the dry state. The two forms are also distinguished by differences in the time after injection at which the greatest number of positive responses appear. The A form is slower and more prolonged in its action. The results in Butenandt and Störmer's recent paper make the occurrence of two forms of keto-hydroxy oestrin more certain. The difference of physiological activity between the two forms is seen in the table. The α -form is about six or seven times more active than the β -form. Butenandt is not certain of the possibility of interconversion of the α - and β -forms. He reports that the α -form occurs only in human pregnancy urine and that most of the oestrogenic activity in the urine of pregnant mares is of the β -form. Certain difficulties arise when one compares the results from different laboratories. Thayer and MacCorquodale (35), working in Doisy's laboratory, have recently shown that by Marrian's technique of assay on the mouse, theelin after keeping for from one week to eight months assays at less than 0.02 γ per unit. This is as potent as Butenandt's α -form. D'Amour and Gustavson (4, 5) who prepare their product by distillation at 0.02 mm. and 130-140° C. should, under these conditions, obtain the α -form, obtain a product which shows the same unit on the mouse and rat and gives for both a unit of 0.38 γ . Butenandt (11) finds that for a single injection in oil the α -form of keto-hydroxy oestrin causes a much less prolonged response in the animals than does the tri-

hydroxy form. Marrian (26), on the other hand, finds that the α -form causes a very prolonged response which occurs after a long latent period when he injects an aqueous solution in four doses over 36 hr. and that the trihydroxy form gives an earlier and shorter response. In this laboratory, on injecting an aqueous solution in six doses in 36 hr., it has been found that theelol and emmenin give a reaction which in its time relations resembles the results of Marrian on trihydroxy oestrin. The reaction with theelin begins slightly later and lasts slightly longer. The latent period and duration in the case of theelin however are not comparable to those obtained by Marrian on a keto-hydroxy oestrin. In 1931 Collip (17) reported the isolation by the present author of a crystalline substance from human placenta using Marrian's method described for pregnancy urine. These crystals showed a similar melting point to Marrian's preparation but were found to have a very low potency when assayed on the adult castrate rat by injection and to be active in doses of 1 γ in immature rats by oral administration. For the trihydroxy form Butenandt and Hildebrandt (10) found that crystals prepared according to Marrian's method (24, 25), melting at 269° C., showed a weight for a unit of 0.68 γ when given in a single injection and 0.12 γ when given in four injections. This last value is in good agreement with Marrian's own value of 0.13 γ shown in the table. However by another method Butenandt was able to prepare a substance having the same elementary analysis and group analysis as the first preparation. This form of trihydroxy oestrin melted at 276° C. or slightly higher than Marrian's preparation. However no lowering of melting point was obtained when the two preparations were mixed and they showed the same specific rotation. The preparation melting at 276° C. showed a weight of 5-10 γ for a unit, or was only one-seventh to one-twelfth as active as the first preparation. Recently Butenandt and Störmer (11) have obtained further results on trihydroxy oestrin. The lower the melting point of the preparation the higher its potency. This they have shown to be due to the contamination of the trihydroxy form with the keto-hydroxy form. Removing the keto-hydroxy form by formation of the semi-carbazone, which is insoluble under the conditions of their experiments, they have isolated from these various preparations a substance which has a potency of 75,000 mouse units per gram (13.3 γ for a unit). The melting point of this substance has been raised to 279° C. (uncorrected). This substance they regard as the pure hydrate uncontaminated with the keto-hydroxy form. In a preliminary report by Collip, Browne and Thomson (18) the possibility of a conversion of the emmenin and theelol to some more active form of oestrogenic substance within the organism and the influence of the ovary in facilitating this conversion was discussed. From the time relations of the animal responses to the pure hydrate Butenandt and Störmer also suggest that even the low potency remaining is due to its partial conversion in the organism to the keto-hydroxy form. Butenandt believes that most of the preparations of trihydroxy oestrin have been mixed with the keto-hydroxy form. As far as the melting point goes there is little difference in potency between that melting at 279° C. and that at 273° C. especially

when the small number of animals used and the percentage response is considered. Moreover Marrian (26, 27) has raised the melting point of his preparation, using an Anschutz thermometer, to 280° C. without reducing its potency. Thayer and MacCorquodale (35) were unable to change the potency of theelol by treatment with hydroxylamine to form the oxime of any theelin present (the oxime as shown by Butenandt has a low potency). They were unable to detect the presence of any theelin in theelol when they attempted to form the semicarbazone of the former. This contrasts with the impure forms of trihydroxy oestrin from which Butenandt readily isolated the semicarbazone of any keto-hydroxy form present. It is difficult to compare Doisy's assays on theelol with those of Butenandt on the hormone hydrate. Doisy's latest figure for theelol, reported at the meeting of the American Society of Biological Chemists in April, 1932, is a unit of about 6.00 γ when injected three times in 12 hr. in the adult ovariectomized rat requiring a squamous response in 75% of the animals. The possibility of the existence of trihydroxy oestrin in more than one form is admitted by Butenandt.

Experimental

In 1930 Collip (14, 15, 16) reported the presence in a crude extract of human placenta of a substance having chemical and physiological properties differing from those of oestrin as then known. This oestrogenic principle was characterized by its non-extractability by ether from acid aqueous solution and by its inactivity in producing oestrus in the adult ovariectomized animal when given by injection. It was, however, very effective when given by mouth to immature intact rats. Later, by using a larger number of animals and dividing the dose into several injections over a period of 36 hr. it was found that the substance was active in producing oestrus when given in large amounts to adult ovariectomized animals but was effective in a fraction of this dose when given to immature intact animals either by feeding or by injection. This oestrogenic substance was given the name emmenin. In the course of its preparation thorough extraction of the solution was performed in order to remove all the ether-soluble oestrogenic substances from the ether-insoluble emmenin. The present author, using the ether extract obtained in this manner, was able to isolate a crystalline substance by a procedure which followed that of Marrian (24, 25) for the preparation of trihydroxy oestrin from pregnancy urine (17, 18). The properties of this crystalline substance obtained from human placenta, the evidence for its identity with the active principle responsible for the emmenin type of oestrogenic activity and a comparison of its physiological properties with those of other crystalline substances, theelol and theelin, will be discussed in this paper.

Throughout this article the placental crystalline substance will be referred to, for convenience, as "emmenin". But if our suggestion that these crystals are a pure trihydroxy oestrin is acceptable, the name emmenin becomes redundant in this connection, though it may still fittingly be employed to

refer to the original crude ether-insoluble fraction, in which, it is believed, the trihydroxy oestrin occurs in combination with some substance unknown which affects its solubility.

Chemical and Physical Properties

Through the kindness of Drs. Graham and Osborne of the Department of Mineralogy a partial crystallographic examination of this preparation was carried out. The system of the crystals was monoclinic, the habit bladed, and they were optically positive. The crystals show an inclined extinction of 7° . Approximate refractive indices were α 1.54, γ 1.68. The crystallographic properties of Doisy's theelol as determined by Slawson (32) are quite comparable with these except that the crystals seem to be optically negative.

The best preparations of the crystalline emmenin melt fairly sharply at 274° C. (uncorrected) with preliminary darkening at 270° C. Through the kindness of Dr. Doisy in supplying a sample of his theelol it has been possible to compare it with emmenin. The sample of theelol melted at 276° C. with slight preliminary darkening. A mixture of equal parts of the two preparations melted at 275° C. (uncorrected). The carbon-hydrogen analysis of two samples of emmenin was: C, 74.8; H, 8.8 and C, 74.8; H, 8.9% respectively.* The crystals are soluble to about 0.05% in boiling water. As far as this chemical evidence goes these crystals appear to be very closely related to theelol. Theelol and emmenin however differ widely in their physiological properties.

We are deeply indebted to Dr. Doisy for supplying us with samples of theelol and theelin, the physiological properties of which we have thus been enabled to compare with those of emmenin.

The probable identity of the crystalline substance with the active principle present in the crude ether-insoluble emmenin fraction is indicated by the following considerations.

Dr. Collip has been able by autoclaving the crude emmenin fraction, obtained by precipitation with ammonium sulphate, at 150 lb. pressure for several hours, to render the active material entirely ether-soluble and to isolate crystals from the emmenin fraction of pregnancy urine by treatment of the ether extract so obtained. These crystals are indistinguishable physiologically from those obtained from placenta by the author. The crystals from pregnancy urine melted at 275° C. (uncorrected) and gave the same carbon-hydrogen analysis. Dr. Collip has also been able to prepare theelol from pregnancy urine. These crystals showed the same physiological properties as those of the samples supplied to us by Dr. Doisy.

The theelol, theelin, and crystalline emmenin from human placenta were compared as to their effectiveness in producing vaginal oestrus in the white rat. Various classes of animals were used.

1. The adult ovariectomized rat.
2. The immature intact rat.

*Research Service Laboratories, New York.

3. The immature intact rat (using oral administration).
4. The immature ovariectomized rat.
5. The immature ovariectomized rat with two immature ovaries transplanted into the spleen.

In assaying emmenin the total amount was injected subcutaneously in aqueous solution in six doses over 36 hr. A full squamous smear or one in which the number of squamous cells greatly exceeded the number of nucleated epithelial cells was called a squamous response. The number of animals showing an absence of leucocytes and the presence of large numbers of nucleated epithelial cells was noted and this number added to those showing a squamous response. The percentage of rats showing either a squamous or an epithelial response is given in the last column of the tables. The definition of these conditions of assay is important since variations in the number of injections and in the criterion of a positive response make great differences in the results obtained.

The Adult Ovariectomized Rat

Table II shows the comparison of the action of emmenin, theelol and theelin using the adult ovariectomized animal as the test object. The dose of

TABLE II
COMPARISON OF EFFECTS OF CRYSTALS OF EMMENIN AND CRYSTALLINE THEELOL AND THEELIN ON ADULT OVARECTOMIZED RATS BY INJECTION SIX TIMES IN 36 HR.

Group	Treatment	Dose γ	Total number of animals	Number of animals giving response indicated			Per cent response	
				Full sq.	Epith.	Neg.	Sq.	Sq. and epith.
1. Old castrates	Emmenin 15	11.0	28	6	1	21	21	25
	Emmenin 10	13.5	21	7	6	8	33	62
	Emmenin 15	20.5	28	15	6	7	54	75
	Emmenin 15	20.5	12	9	1	2	75	83
	Emmenin 15	14.6*	13	0	4	9	0	31
	Theelol	2.5	15	8	2	5	53	67
		3.0	14	6	1	7	30	50
		5.0	21	17	3	1	81	95
2. Recent castrates	Emmenin 15	15.7	100	62	20	18	62	82
	Theelol	1.57	100	80	8	12	80	88
3. Sensitized castrates	June 13 Theelol	1.44*	47	6	3	38	12	19
	June 20 Theelol	1.72	46	31	3	12	70	75
	June 27 Emmenin 15	7.20	40	8	12	20	20	50
	July 4 Emmenin 20	7.50	46	17	9	20	37	56
	July 12 Theelol 2	1.68	44	25	2	14	57	61
4. Recent castrates (rats not used previously)	May 30 Theelin	0.15	22	0	0	22	0	0
	June 6 Theelin	0.36	17	1	0	16	6	6
	June 13 Theelin	0.90	25	22	0	3	88	88
	June 20 Theelin	0.40	29	27	1	1	93	96
	June 27 Theelin	0.15	29	0	0	29	0	0
	July 4 Theelin	0.30	29	5	1	23	18	21

*These rats had not been used for a month before this experiment.

emmenin required for a given response varies according to the condition of the animal. The first group of rats had been ovariectomized some months before these assays and had been used from time to time. All the groups except that on the 14.6 γ dose had been primed with theelol before use. The lower response of the unprimed rat is evident. From these assays emmenin seems to be about one-fifth as active as theelol. In Group 2, a large group, the animals had been ovariectomized two weeks before the first emmenin assay and no previous priming dose was given. One week later the dose of 1.57 γ of theelol produced an 80% squamous response. In this assay emmenin is less than one-tenth as active as theelol though the unit for both is less than in the case of the old castrates. The objection might be raised that the first dose of emmenin sensitized the rats to the theelol and that if the theelol had been given first the dose for both would have been the same for both substances. In Group 3 the influence of six weeks rest on the response of the animals is seen. These are part of the group of 100 mentioned above. One week later a 70% squamous response was obtained with 1.72 γ of theelol. The next week 7.20 γ of emmenin gave a 20% response and another preparation of emmenin a 37% response. Finally a second preparation of theelol, also kindly supplied by Dr. Doisy, gave a 57% response with 1.68 γ on the same group of animals.

The above experiments show that emmenin is approximately one-fifth as active as theelol when tested on the adult ovariectomized animals under these conditions of assay. This physiological difference contrasts with the close chemical relation. The results with theelin are incomplete but indicate the effect of a previous larger dose on sensitizing the animals to a subsequent much smaller dose. These rats were recently castrated and were used only for theelin assay.

The Immature Intact Rat

The immature intact rat of 21 days of age responds in quite a different manner. The results as shown in Table III demonstrate that emmenin acts in a much lower dose in the immature intact animal than in the adult ovariectomized animal, that theelol acts in the same dose in both types of animal, and that emmenin and theelol are equally effective on the immature rat. There is considerable individual variation in the response of different groups of animals. Emmenin preparation No. 20 (m.p., 270° C.) seems slightly more active than preparation No. 15 (m.p., 274° C.). It was also slightly more active on the adult ovariectomized animal.

The effect of theelin on the immature rat is seen in the third part of Table III. It seems to be slightly more effective than emmenin or theelol but if one compares emmenin No. 20 and theelin, 0.92 γ of the emmenin gives a 33% response, 0.90 γ of theelin gives a 50% squamous response and 1.40 γ of emmenin gives a 100% response as does 1.20 γ of theelin. Compare this with the action on the adult where after a previous sensitizing dose in each case emmenin No. 20 gave a 37% response on 7.5 γ whereas theelin gave a 93% response on 0.40 γ . In other words emmenin was about one twenty-fifth as active as theelin in the adult ovariectomized rat.

TABLE III

COMPARISON OF EFFECTS OF CRYSTALS OF EMMENIN AND CRYSTALLINE THEELOL AND THEELIN ON IMMATURE INTACT RATS BY INJECTION SIX TIMES IN 36 HR.

Treatment	Age	Dose γ	Total number of animals	Number of animals giving response indicated			Per cent response	
				Full sq.	Epith.	Neg.	Sq.	Sq. and epith.
Emmenin								
Ext. 20	22	0.92	6	2	1	3	33	50
15	22	1.13	10	0	0	10	0	0
15	22	1.21	13	7	3	4	54	70
15	22	1.32	12	3	1	8	25	33
20	22	1.40	13	12	1	0	93	100
15	22	1.55	30	14	4	12	47	60
15	22	1.65	10	0	1	9	0	10
10	22	1.80	6	3	1	2	50	67
Theelol								
	22	1.25	6	3	0	3	50	50
	22	1.62	9	4	5	0	45	100
	22	2.50	6	4	0	2	68	68
Theelin								
	22	0.45	10	0	0	10	0	0
	22	0.60	16	1	3	12	6	25
	22	0.60	15	4	2	9	26	40
	22	0.90	6	3	0	3	50	50
	22	1.20	9	9	0	0	100	100
Effect of repetition of the dose								
	24	0.60	16	1	3	12	6	25
	31	0.60	16	10	1	5	62	69

The Immature Intact Rat (Oral Administration)

The activity of emmenin and theelol is also the same when they are given orally to the immature intact rat as shown in Table IV. The total dose was given over three days and was placed in the drinking cup so that a slow, almost continuous absorption took place. In agreement with the finding

TABLE IV

COMPARISON OF EFFECTS OF CRYSTALS OF EMMENIN AND CRYSTALLINE THEELOL AND THEELIN ON IMMATURE INTACT RATS. EFFECT OF ORAL ADMINISTRATION

Treatment	Dose γ	Total number of animals	Number of animals giving response indicated			Per cent response	
			Full sq.	Epith.	Neg.	Sq.	Sq. and epith.
Emmenin 15	0.92	10	3	3	4	30	60
15	1.00*	20	2	3	13	10	35
10	1.26	9	5	2	2	55	75
10	1.40	6	3	1	2	50	67
10	1.80	5	3	0	2	60	60
15	2.00*	20	3	2	13	15	35
Theelol							
	1.32	10	3	0	7	33	33
	1.50	5	5	0	0	100	100

*These assays were on the same group of animals.

that division of the dose into parts increases the effectiveness of oestrogenic substances both emmenin and theelol are more effective when administered by mouth in this way than by injection when the dose is given in six parts in 36 hr. Variation in groups of rats is again seen in this table.

The Immature Ovariectomized Rat

Because of weight differences and possible differences in absorption from the subcutaneous tissue the comparison between adult ovariectomized animals and immature intact animals is perhaps not quite valid. The use of the immature ovariectomized animal of about the same age as the intact normal used above overcomes this difficulty. The results are strikingly different from those with the immature intact animal, and are shown in Table V. The dose of emmenin required is about the same or even slightly greater than that for a corresponding response in the adult ovariectomized animal. The dose of theelol required for the immature ovariectomized rat is about one and a half times that for the immature intact rat.

TABLE V

COMPARISON OF EFFECTS OF CRYSTALS OF EMMENIN AND CRYSTALLINE THEELOL AND THEELIN ON IMMATURE OVARECTOMIZED RATS BY INJECTION SIX TIMES IN 36 HR.

Treatment	Age at castration	Age at injection	Dose γ	Total number of animals	Number of animals giving response indicated			Per cent response	
					Full sq.	Epith.	Neg.	Sq.	Sq. and epith.
Emmenin 15	21	23	4.4	11	1	0	10	9	9
	21	23*	6.2	11	2	2	7	18	36
	21	23	9.5	10	2	3	5	20	50
	21	29*	10.0	11	4	1	6	36	45
	21	50	10.2	15	11	2	2	73	87
	21	23	12.0	10	7	1	2	70	80
Emmenin 10	28	31	6.0	8	3	1	4	37	50
Theelol (repeated doses)	21	26	2.43	20	0	1	19	0	5
		32	3.24	20	11	2	7	55	65
	19	21	1.62	6	1	0	5	16	16
		32	2.43	12	6	2	4	50	66
Theelin (repeated doses)	22	26	0.30	7	0	0	7	0	0
	22	24	0.60	13	2	3	8	15	38
	22	26	0.60	8	2	0	6	25	25
	22	26	0.60	8	2	0	6	25	25
		30	0.75	8	7	0	1	89	89
	22	26	0.30	7	0	0	7	0	0
		30	0.75	7	7	0	0	100	100
	21	23	0.60	13	2	1	10	15	23
		35	0.60	13	11	0	2	84	84
		42	0.40	13	4	2	7	31	46

*This is the same group of rats.

Thus the difference between the activity of emmenin on the adult ovariectomized and immature intact rat is not related to weight differences or differences in absorption rate in the two types of test object. The difference in response in the immature intact and immature normal animal suggests that the presence of the immature ovary plays some role in the greater sensitivity of the normal animal. That the operation itself has no effect on the response of an animal to emmenin is shown by a control experiment where six animals were operated upon at the age of 21 days. The uteri were pulled on and cut but the ovaries were not removed. These animals showed a 100% squamous response on 1.5 γ of emmenin No. 15.

Theelol appears to be slightly more effective in the presence of the ovary but the difference is not nearly as great as in the case of emmenin.

The difference of effect between the normal and ovariectomized immature rats tends to decrease as the number of injections into which the total dose is divided is increased. Thus when nine injections in 48 hr. are given, a dose of 6.2 γ of emmenin is sufficient to give an 88% squamous response. And when the extract is fed over three days by the method mentioned above there is no difference between the response of the ovariectomized and of the intact immature animal.

The effect of theelin on the immature ovariectomized animal is the same or greater than its effect on the intact rat of the same age when it is injected in six doses over 36 hr. The sensitizing action of one dose of theelin on the next is very marked. Thus one group of 13 animals gave a 15% squamous response to 0.60 γ . Twelve days later they showed an 84% response to the same dose, and one week later a 31% response to 0.40 γ . In the case of emmenin this sensitization is not seen, a group of 11 immature ovariectomized rats showing an 18% response to 6.2 γ and six days later a 36% response to 10.0 γ . This is only the increase to be expected with the increase in dosage.

The Immature Ovariectomized Rat (With Two Immature Ovaries Transplanted)

In an endeavor to elucidate further the possible role of the ovary in the change of sensitivity to emmenin observed when the immature rat was ovariectomized, transplantation experiments were undertaken. The rats were castrated, usually at 21 days of age, tested for their response to one of the oestrogenic substances, and six days later received two ovaries from 21-day-old rats. These were transplanted into the spleen of the recipient. Two days later the rats were again tested with the same oestrogenic substance as before. The results are shown in Table VI.

The transplantation of the ovaries into immature ovariectomized rats lowered the dose of emmenin and theelol required for a given response to the level for the immature intact animal. This is further evidence for the theory that the immature ovary plays a specific role in the difference in response to emmenin observed in the ovariectomized and intact animal. That implantation of the ovaries alone does not produce a positive reaction is shown by the results on a group of nine 23-day normal rats which received two adult ovaries in the spleen. The smears were followed daily, one showed a positive

TABLE VI

COMPARISON OF EFFECTS OF CRYSTALS OF EMMENIN WITH CRYSTALLINE THEEOL AND THEELIN ON IMMATURE RATS. TRANSPLANTATION EXPERIMENTS

Treatment	Test animal	Age	Dose γ	Total number of animals	Number of animals giving response indicated			Per cent response	
					Full sq.	Epith.	Neg.	Sq.	Sq. and epith.
Emmenin	Group 1 Castrated	19							
		23	6.2	11	2	2	7	18	36
		28	10.0	11	4	1	6	36	45
	Transplanted	30							
		34	2.7	11	10	1	0	91	100
		41	1.8	11	7	3	1	63	91
Emmenin	Group 2 Castrated	21							
		24	9.5	10	2	3	5	20	50
		27							
	Transplanted	30	2.3	10	7	2	1	70	90
		36	1.56	10	6	1	3	60	70
Emmenin	Group 3 Castrated	21							
		23	12.0	10	7	1	2	70	80
		26							
	Transplanted	29	1.35	10	8	0	2	80	80
Theelol	Group 1 Castrated	23							
		26	2.43	20	0	1	19	0	5
		32	3.24	20	11	2	7	55	65
	Transplanted	35							
		37	1.72	20	15	1	4	75	80
Theelin	Group 1 Castrated	21							
		26	0.60	15	4	2	9	26	40
		28	0.60	14	12	1	1	86	93
	Transplanted	34							
		36	0.40	13	10	2	1	77	94

smear at the age of 51 days, five were cyclic at 60 days of age. Ten immature rats were ovariectomized at the age of 19 days and received two ovaries from 19-day-old rats when the recipients were 28 days old. The smears were read daily. No positive smear was noted up to three weeks after the implantation. The rats were 85 days old when the readings were stopped and only one was cyclic. This may be due to degeneration of the grafts or to the rats being still immature. In this connection one of a group of rats which had been implanted was killed a month after implantation. The rat was acyclic and the implanted ovaries were visible on the spleen. The graft weighed 14 mg.,

the original implant 7 mg. The graft consisted apparently of immature follicles. Another group of ten rats was ovariectomized at 20 days, transplanted with 21 day ovaries at 27 days and the smears followed daily. One showed a positive smear at the age of 40 days and remained cyclic, and one became cyclic at the age of 84 days. The results indicate that the transplantation of immature ovaries into immature ovariectomized rats will not of itself cause a positive smear within the term of the transplantation experiments described above. The results of Martins and Rocha (30) on parabiosis of castrate immature males with immature normal females, where the immature female became cyclic, indicate that the immature castrate male shows certain changes (presumably in the anterior pituitary) which stimulate an immature ovary brought under the influence of substances in the circulation of such an animal by parabiosis. It was thought that the transplantation results might be accounted for on the basis of changes occurring in the pituitary of the immature female after castration—such as the occurrence, when the ovaries were later implanted into the animal, of a premature stimulation of these ovaries with the production of an oestrus cycle.

The mechanism of action of the immature ovary in producing this increase of sensitivity to emmenin is not clear. The immature ovary might contribute part of the amount of oestrogenic substance required from its own store of this type of substance either because at this period after implantation the graft has not fully taken and part of the implant is being resorbed or because, the graft having taken, the immature ovary is being stimulated to secrete a certain amount of oestrin into the circulation. It has been shown in this laboratory that macerated immature ovaries implanted into the adult ovariectomized animals produced a positive response in a dose of 40 mg. The usual weight of two immature ovaries is 7-10 mg. The possibility of a pre-pubertal secretion of oestrin even in the intact rat has been noted by Mirskaia and Wiesner (31). According to this point of view the dose of emmenin is less for both the immature intact and the immature implanted animal because the ovary is in each case contributing part of the required amount of oestrogenic substance and hence the amount which must be injected is less. A control experiment was performed in this connection. Ten immature rats 21 days old were implanted with two extra ovaries from 21-day-old rats. They were injected two days later with 0.83 γ of emmenin 15, which on ordinary immature rats showed a 54% positive response on 1.21 γ . One of the rats with the extra ovaries showed an epithelial response and the rest were negative. The presence of two extra ovaries does not therefore make the rats more sensitive to emmenin as would be expected if the implanted ovaries were contributing some of the oestrogenic substance required. The fact that the dose of theelin required for the immature normal rat is the same as for the immature ovariectomized rat is also against the view that the effect of transplantation on the sensitivity to emmenin is due to the immature ovary contributing a part of the dose of oestrogenic substance required. The effect of transplantation on the dose of theelin required is not so easy to determine on account of the marked sensitizing effect of one dose of theelin

on the next. One cannot tell whether any increase in sensitivity of the rats after transplantation is due to the effect of the transplantation or to that of the dose of theelin given before transplantation. Further experiments are needed in this connection. A group of 15 animals was given 0.06 γ of theelin at the age of 21 days, and showed a 26% response. They were ovariectomized at the age of 26 days and two days later were tested again with 0.60 γ . They showed an 86% positive response. This may be compared with the increase in sensitivity on repeating the dose of 0.60 γ of theelin on another group of 16 rats which were not ovariectomized between the tests but where the interval between the assays was the same. In the latter group the first test gave a 6% and the second a 62% positive response. There is no significant difference between the two groups so that ovariectomy seems to make little difference to the increase in sensitivity due to a previous dose of theelin. The absence of effect of ovariectomy is also seen in the last group in Table VI. The rats in this group were ovariectomized before the first dose of theelin. The first group of animals received two 21-day-ovaries at the age of 34 days. Two days later they showed a 77% positive response with 0.4 γ of theelin. This result may be compared with the last group in Table VI. Here the response of animals ovariectomized at 21 days was 15 to 0.60 γ at 23 days of age; at 35 days of age it was 84% to the same dose, and at 42 days of age 31% to 0.40 γ . In this case the transplantation of two ovaries seems to have increased the sensitivity of the animals in the first group as compared to that of the second group which received the same number of doses of theelin at the same intervals previously, but in which no transplantation was performed. The increase in sensitivity is slight, however, when compared with that occurring when emmenin is used on animals before and after transplantation and the number of animals is too small for any conclusion to be drawn.

Adult ovariectomized animals have also been used for transplantation experiments. Immature ovaries were used. The smears were followed daily. The danger of the occurrence of spontaneous oestrus makes these results less reliable than those on the immature animal. A large number of the old adult animals thus implanted remain acyclic. However most of them now gave a positive response to the immature normal dose of emmenin (1.5 γ) where before they required 7-16 γ for a similar response. This is taken to indicate that the presence of the implanted ovary, even though the graft may from outward appearances have degenerated (since no cyclic phenomena are produced), is still capable of making the animals respond to the immature normal dose of emmenin.

While there are still many obscure features it is felt that the theory which at present is most tenable, in view of the above considerations, is that the immature ovary may convert emmenin and probably theelin to some more active form of oestrogenic substance (probably some form of keto-hydroxy oestrin). This conversion is not impossible in the absence of the ovary but the rate of conversion and of utilization of emmenin is greatly increased in

its presence. The adult ovary also may presumably perform this conversion but it is not necessary that the ovary be producing cyclic changes for this conversion to occur.

The length of time since transplantation in the above experiments, necessarily limited because of the danger of spontaneous puberty later, makes it uncertain whether any effect is due to a secretion by the implanted ovary in the true sense after the graft has taken, or whether it is due to the substances which effect the conversion of emmenin being liberated from the graft before it has actually taken. The experiments with the adult implanted animals indicate that even after some weeks, when presumably a blood supply has been established, the conversion effect takes place.

The latest work of Butenandt (11) shows that trihydroxy oestrin uncontaminated with the keto-hydroxy form has a potency, when assayed on the adult castrate mouse by a single injection in oil, of 75,000 units per gram, requiring 80% approximately of the test animals to show a positive response. It is very difficult to compare this with the values with emmenin because of assay differences.* Emmenin has a potency of 50,000-70,000 units per gram when an 80% positive response is required, the adult ovariectomized rat being used and the dose divided into six injections in water over a period of 36 hr. The results reported in this paper make it probable that even the low potency of the trihydroxy form which remains after removal of the keto-hydroxy form is due to a conversion of the trihydroxy into the keto-hydroxy form in the organism, and that the presence of even an immature ovary facilitates this conversion. The problems as to which of the forms of keto-hydroxy oestrin (A or B) is secreted by the ovary in the organism under physiological conditions, whether they are interconvertible in the organism and under what conditions the organism is capable of converting the keto-hydroxy form into the trihydroxy form are of physiological significance. The occurrence in large amounts of the trihydroxy type in pregnancy urine in the human (10) makes the last process probable.

Summary

1. A crystalline oestrogenic substance has been isolated from human placenta. Evidence is presented for the identity of this substance with the active principle of the ether-insoluble compound previously demonstrated by Collip in a crude extract of human placenta and called by him "emmenin". The melting point of this substance is 274° C. (uncorrected). Analysis of two samples showed C, 74.8; H, 8.8% and C, 74.8; H, 8.9%. The crystal form has varied. The crystals are monoclinic, optically positive and show approximate refractive indices of α 1.54 and γ 1.68 and an inclined extinction of 7°. The solubility of the crystals in water at 100° C. is about 0.05%.

**The author has recently had the opportunity of making a direct comparison of the two substances in Dr. Butenandt's laboratory; emmenin (preparation No. 20) proved to be somewhat more active in the adult ovariectomized mouse than Butenandt's uncontaminated hormone-hydrate. Meanwhile a sample of the latter, kindly supplied by Dr. Butenandt, has been found at McGill University to be of the same order of potency as theelol and the emmenin crystals in the intact immature rat.*

A mixed melting point of the compound with a sample of crystalline theelol gave no significant lowering of the melting point. The theelol melted at 276° C., the emmenin at 274° C., and a mixture of equal parts of both at 275° C. All values are uncorrected.

2. Emmenin was assayed on the adult ovariectomized white rat. The total dose was given in aqueous solution in six injections over 36 hr. The response of the animals varied according to their condition. A dose of about 15 γ was required to cause a squamous response in 50% of the animals used. The dose of theelol required for a similar response also varies but this substance was five to ten times more active than emmenin when tested on the same group of animals under the same conditions.

3. The dose of emmenin required for the immature intact rat of 21 days of age varies from 0.92 γ to 1.80 γ for a squamous response in 50% of the test animals when injected in six doses over 36 hr. The dose required when the emmenin is fed over a period of three days tends to be slightly lower than when injected as above. The dose of theelol for the immature intact rat is the same as that of emmenin both for feeding and injection experiments.

4. The dose of emmenin required for a 50% squamous response in the immature ovariectomized rat two to four days after ovariectomy was from 7 to 10 γ or about eight times that required for the immature normal animal of the same age. When tested on the immature ovariectomized animal the dose of theelol was about one and a half times that for the normal rat of the same age, that is about 2 to 3 γ . This increase is slight when compared to that taking place in the case of emmenin.

5. Transplantation of ovaries from 21-day-old rats into the spleen of immature ovariectomized rats causes the dose of emmenin necessary for a given response to fall from the level required for immature ovariectomized animals to that required for the immature intact animal. This fall in the dose occurs in the case of theelol also.

6. The activity of crystalline theelin when tested on the immature normal rat is nearly the same as that of emmenin. There is no change in sensitivity to theelin on ovariectomy of the immature animal. Transplantation of ovaries into the previously ovariectomized immature rat produced little change in sensitivity to theelin. The first dose of theelin sensitizes both ovariectomized and normal animals to a subsequent dose of the same material.

7. The theory advanced to account for these findings is that the immature ovary increases the efficiency of utilization of emmenin, and probably of theelol, by increasing the rate of conversion of these substances to a more active form of oestrogenic substance (probably some form of keto-hydroxy oestrin). This conversion can occur in the absence of the ovary, though much less effectively. It is not necessary that the ovary be producing cyclic phenomena for this conversion to take place.

Acknowledgment

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STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES.

XLV. THE POLYMERIZATION, UNDER THE INFLUENCE OF HEAT, OF CERTAIN COMPOUNDS CONTAINING THE ETHYLENE OXIDE RING¹

By EDWIN C. JAHN² AND HAROLD HIBBERT³

Abstract

The tendency of certain glycidol and glycoside derivatives to polymerize under the influence of heat has been investigated. Phenyl glycidol, methyl glycidol, and 2 : 3, 6 : 7-di-epoxy-4, 5-dihydroxy octane all undergo polymerization. With phenyl glycidol acetate the tendency is much less pronounced, while polymerization does not occur with phenyl glycidol methyl ether. Apparently the phenomenon is associated with the presence of a hydrogen atom capable of migration. Polymerized phenyl glycidol was found to have an open-chain structure containing one ethylene oxide ring. 2-Methoxy ethyl glycoside was obtained as a stable dimer, which appears to have a dioxane structure.

Introduction

In order to obtain a better understanding of the structure of cellulose and other polysaccharides, it seemed desirable to determine the manner in which simpler compounds containing *typical groups* unite to form larger molecules.

The hypothesis has been advanced that polysaccharides are derived from a simple building unit through the medium of residual valence forces, the seat of which lies in the oxygen ring (1, 2, 5, 19, pp. 576-604, 20). On the other hand rather convincing evidence has been presented that certain carbohydrate derivatives, for example 2, 3, 4-trimethyl arabonolactone, polymerize as a result of ring fission of an unstable oxygen ring, this leading to molecular combination by means of normal valency forces (12). Experimental evidence indicates that inulin is derived similarly from anhydrofructose units (17). This "normal valency" theory of polymerization has been extended to include all polysaccharides (14, 15, 16, pp. 74-96, 29, 30, 31).

It is not impossible that each of these factors may play a part in the formation of polymers from oxygen ring compounds. Variations in the structure

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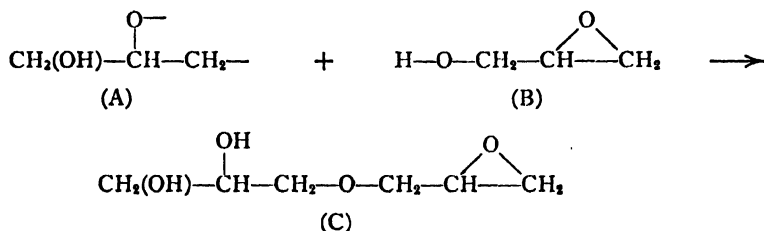
of the molecule, other than the nature and size of the ring, may also have a determining influence on the tendency towards polymerization, or association, and on the mechanism of the process.

Levene and Walti (24-28) studied various glycidol derivatives having certain constitutional relationships to the sugars, in an attempt to obtain data capable of being applied to an interpretation of the structure and mechanism of formation of the natural polysaccharides. No generalizations can be made as yet, however, other than that in the individual cases examined, differences in mode of combination occur, due probably to variations in molecular structure having a direct influence on ring stability.

Glycidol, $\text{CH}_2\text{—CH—CH}_2\text{—OH}$, when heated in a sealed tube, was found by



Nef (32) to undergo transformation into a resin. He assumed that this complex product was a polymerized glycidol chain compound formed as follows:



(C) then undergoes ring fission, and combines with another molecule of (B), the process thus giving rise to a chain polymer containing one terminal ethylene oxide ring.

The recent work of Levene and Walti (24, 25, 27) on propylene oxide, glycidol and glycidol acetate, has provided experimental evidence in support of this view.

In the earlier literature dealing with the simple carbohydrate type of compounds such as glycollic aldehyde, acetol, glyceric aldehyde, and dihydroxyacetone, Fenton (13), Bertrand (9, 11), Wohl (34, 35), Nef (32) and Kling (23) presented evidence indicating an equilibrium between the monomolecular and bimolecular forms of these compounds.

Investigations by Bergmann and coworkers (1-7) on the molecular weight of various lactolides, such as the methyl lactolide of acetol; ethyl glycoloside; glycolaldehyde-lactol acetate; methyl lactolide of cyclohexanol (2)-on-(1); acetaldol-lactol acetate, etc., led to the conclusion that while these products in organic solvents had a molecular weight corresponding to a dimer, in the gaseous state, judged in the light of their vapor density determinations, they appeared to be monomolecular. This passage from the monomeric to the dimeric stage, and its converse, were assumed to take place under the influence of "residual" rather than "normal" valency forces. However, in a later paper, Bergmann and Miekeley (8) were able to show that the earlier

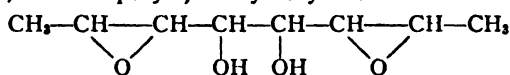
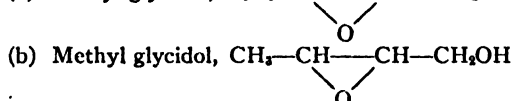
views were incorrect due to the fact that their previous determinations of the vapor densities were inaccurate, and they arrive at the conclusion that all the above products possess a normal valency structure derived from two molecules of the monomolecular form.

Sole reliance upon molecular weight values for determination of structure has been severely criticized (16, pp. 74-96).

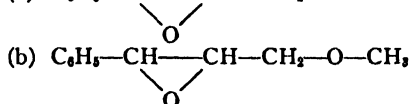
The simple ethylene oxide ring compounds offer an inviting field for study, because of the reactivity of the ring and its simple structure, and also in view of the conflicting data regarding ring behavior during transformation to the supermolecular state. It is necessary to collect further evidence regarding the nature of polymerization of this class of compounds before any generalizations can be made.

In the present paper the tendency towards polymerization, or association, under the influence of heat, of the following compounds was studied:

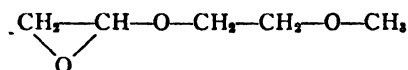
I. Analogues of Glycidol



II. Phenyl Glycidol Acetate and Methyl Ether



III. 2-Methoxy Ethyl Glycoloside



Discussion of Results

POLYMERIZATION OF GLYCIDOL ANALOGUES

Phenyl glycidol, methyl glycidol and 2 : 3, 6 : 7-di-epoxy-4, 5-dihydroxy octane contain a free hydroxyl group in the same position relative to the oxide ring. They should, therefore, exhibit a similar tendency to polymerize under the influence of heat, as shown by glycidol, and this was found to be the case.

2 : 3, 6 : 7-Di-epoxy-4, 5-dihydroxy octane polymerizes more readily than phenyl or methyl glycidol, as would be expected from the "double glycidol" nature of its molecule. This substance could not be distilled unchanged from its reaction mixture due to the ease with which it resinified.

(a) *Phenyl Glycidol*

Phenyl glycidol when heated at 140-155° C. for 29 hr. changed to a very viscous orange-colored liquid which was essentially pure dimeric phenyl glycidol. When heated for a much longer period (32 days) in sealed tubes at 105° C. solid resins were obtained from which a colorless amorphous polymer could be isolated.

Methylation of the dimeric phenyl glycidol yielded a dimethoxy derivative. Addition of ammonia to this dimethoxy compound gave a product formed from one mole of ammonia and one mole of dimethoxy dimeric phenyl glycidol, thus proving the presence of only one ethylene oxide ring. Trityl chloride and the dimeric phenyl glycidol gave a monotrityl derivative (18), indicating the presence of only one primary hydroxyl group, and not two as would be the case, presumably, if the compound were an "associated product".

The addition of ammonia to the solid polymerized phenyl glycidol yielded amines which contained less than one atom of nitrogen per molecule of polymer. This indicated the presence of not more than one ethylene oxide ring, as was also found to be the case by Levene and Walti (24) with polyglycidol.

Polymerization of phenyl glycidol apparently takes place through a primary ring fission of one molecule and addition to this of a second unchanged molecule. The resulting polymer still contains one ethylene oxide ring, which in turn undergoes ring fission and then adds on a further molecule of the original glycidol.

(b) *Methyl Glycidol*

Methyl glycidol is converted into an open-chain dimer when heated at 105° C. in a sealed tube for 22 days. The latter reacts with phenyl isocyanate to form a di-phenyl urethane derivative which still contains an ethylene oxide group, indicating that methyl glycidol most probably polymerizes in the same manner as phenyl glycidol.

(c) *2 : 3, 6 : 7-Di-epoxy-4, 5-dihydroxy Octane*

Attempts to prepare and isolate 2 : 3, 6 : 7-di-epoxy-4, 5-dihydroxy octane by oxidation of dipropenyl glycol with benzoylhydroperoxide yielded almost entirely a resin. The monomeric form was most probably present in the reaction mixture because the theoretical amount of oxygen was consumed for the reaction, but under the influence of heat, it apparently undergoes polymerization very readily. The structure of this polymer has not yet been investigated. The molecular weight of the resin indicated that it was essentially a trimer.

POLYMERIZATION OF PHENYL GLYCIDOL ACETATE AND OF PHENYL GLYCIDOL METHYL ETHER

The tendency for phenyl glycidol, phenyl glycidol acetate, and phenyl glycidol methyl ether to polymerize was found to decrease in the order named. This would seem to indicate an intimate relation between tendency to poly-

merize and the presence, or absence, of a hydrogen atom capable of migrating, since when this is replaced by a methyl group, as in the methyl ether, the tendency to undergo polymerization ceases.

Phenyl glycidol acetate was readily transformed into a very viscous yellow substance by heating for six days at 145-165° C. at atmospheric pressure, or for four weeks in a sealed tube at 140° C. The greater part of the reaction product was a solid red resin, apparently a polymerized phenyl glycidol acetate, having an average molecular weight of 623, and thus corresponding, approximately, to a trimer.

Phenyl glycidol methyl ether, when heated for a long period in a sealed tube with or without a catalyst, yielded only resins, oily liquids and water, indicating that decomposition with loss of water and condensation had occurred. None of the resins formed appeared to be polymers of phenyl glycidol methyl ether.

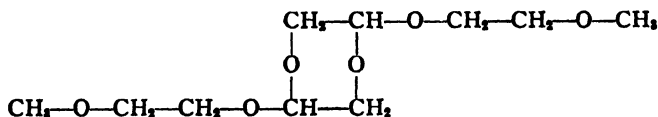
POLYMERIZATION OF 2-METHOXY ETHYL GLYCOLOSIDE

When methoxy ethyl vinyl ether ($\text{CH}_2=\text{CH}-\text{O}-\text{CH}_2-\text{CH}_2-\text{O}-\text{CH}_3$) was oxidized with benzoylhydroperoxide, only the dimeric 2-methoxy ethyl glycoloside (b.p., 118-119° C./0.1 mm.) could be isolated.

Apparently under the conditions of synthesis and distillation, the monomer undergoes dimerization. The dimer formed is quite stable towards heat for it may be redistilled nearly quantitatively (0.1 mm. pressure) without decomposition.

In alcoholic ammonia solution the dimer absorbed only a very small amount of ammonia. This may be due either to the presence of a small amount of an ethylene oxide form present, perhaps in equilibrium with a more stable form, or to the fact that the dimer, in the stable dioxane form, is slightly decomposed allowing addition of a small amount of ammonia.

The fact that no dissociation occurs when the dimer is distilled and that the product combines with only one-sixth of a mole of ammonia, instead of two moles, points to its structure as that of a stable dioxane derivative,



rather than an "associated" type of polymer.

Experimental Part

The oxidation of the unsaturated compounds to ethylene oxide derivatives was carried out in all cases by means of benzoylhydroperoxide (Prileschajew's reagent) (33). The procedure outlined by Hibbert and Burt (21) was followed with slight modifications.

PHENYL GLYCIDOL AND ITS POLYMERS

Preparation of Phenyl Glycidol

To a chloroform solution of benzoylhydroperoxide containing 6.2 gm. of available oxygen was added 49 gm. of cinnamyl alcohol, the temperature of the solution being kept at 0° C. Reaction was complete after 20 hr. Yield of liquid phenyl glycidol, 42.9 gm. (b.p., 134-135° C./4-5 mm.). n_D^{27} , 1.5427. It readily decolorized potassium permanganate solution but not bromine in carbon tetrachloride.

After standing 16 hr. at room temperature, the product solidified to a mass of wax-like crystals; m.p., 26.5° C. Analysis:—Found*: C, 71.86; H, 6.58%. Calcd. for $C_9H_{10}O_2$: C, 72.00; H, 6.66%. Mol. wt. ($C_2H_4Br_2$), 154.6; calcd., 150.

Phenyl Urethane Derivative

This was obtained by mixing together phenyl glycidol and phenyl isocyanate. After recrystallization from benzene-ligroin solution the product melted at 87° C. Found: C, 71.66, 71.77; H, 5.20, 5.47%. Calcd. for $C_{16}H_{15}O_3N$: C, 71.64; H, 5.57%.

Dimeric Phenyl Glycidol

Phenyl glycidol (6.5 gm.) was heated for 29 hr. at 140-155° C. The thick viscous mass so obtained was then heated at 100° C./15 mm., yielding in this way less than 0.1 gm. of distillate.

Analysis of the residual liquid showed it to be practically pure dimeric phenyl glycidol. n_D^{20} , 1.5744. Found: C, 72.08, 72.06; H, 6.77, 6.80%. Calcd. for $(C_{18}H_{20}O_4)$: C, 72.00; H, 6.66%. Mol. wt. ($C_2H_4Br_2$), 328, 309; calcd., 300.

Trityl Derivative

One gram of the dimer was dissolved in 5 cc. of pyridine and 1.86 gm. of trityl chloride added. After 24 hr. the mixture was shaken thoroughly with water, the solid reaction product dissolved in benzene, and the product precipitated by adding ligroin. After three precipitations the trityl derivative was obtained as a micro-crystalline colorless product. Its alcoholic solution was readily decolorized by potassium permanganate. When dissolved in dry toluene and treated with clean sodium an evolution of gas occurred. Mol. wt. ($C_2H_4Br_2$); found, 536; calcd. for the monotrityl derivative of dimeric phenyl glycidol ($C_{37}H_{34}O_4$), 542.

Dimethyl Ether of Dimeric Phenyl Glycidol

The dimer (1.75 gm.), dissolved in 10 gm. of methyl iodide, was mixed with 7 gm. of silver oxide and 3 gm. of anhydrous sodium sulphate, and the mixture kept at 45-50° C. under reflux for 24 hr. The reaction product was filtered and the residue washed with ether. Removal of the ether and methyl iodide, followed by distillation of the residue, yielded 0.6 gm. of a colorless

* The micro-method of analysis was used throughout this investigation.

oil; b.p., 180-185° C./0.03 mm. n_D^{25} , 1.5383. The product readily decolorized permanganate solution. Found: CH_3O , 18.72%. Calcd. for $\text{C}_{20}\text{H}_{24}\text{O}_4$; 18.90%. Mol. wt. ($\text{C}_2\text{H}_4\text{Br}_2$), 314; calcd. 328.

The residue was a reddish viscous oil which could not be distilled. It contained 14.77% CH_3O and hence was only partially methylated.

Addition of ammonia. A portion (0.25 gm.) of the dimethyl ether of dimeric phenyl glycidol was dissolved in 50 cc. of alcoholic ammonia (saturated) and left standing in a sealed bottle for three days at 35-40° C. Removal of the alcohol and excess ammonia by vacuum distillation left a viscous orange-colored material. Found: N, 4.16%; calcd. for $(\text{C}_{20}\text{H}_{24}\text{O}_4 + \text{NH}_3)_x$, 4.06%.

Phenyl Glycidol Polymer

Phenyl glycidol (10 gm.) when heated in a sealed tube at 105° C. for 32 days yielded a solid orange-colored resin. This was purified by dissolving in 50 cc. of benzene and precipitating by the addition of 150 cc. of ligroin, the operation being repeated three times. The white fluffy powder so obtained decolorized cold potassium permanganate solution and in dry toluene reacted with sodium, evolving a gas. Found: C, 71.83; H, 6.90%. Calcd. for $(\text{C}_9\text{H}_{10}\text{O}_2)_x$: C, 72.00; H, 6.66%. Mol. wt. ($\text{C}_2\text{H}_4\text{Br}_2$), immediately after solution, 816; after 30 min., 496; after 24 hr., 438.

Addition of ammonia. The polymerized phenyl glycidol (0.7 gm.) when mixed with 150 cc. of alcoholic ammonia solution and allowed to stand for three days at 30° C., gave a solid yellow resin. Found: N, 0.343, 0.379%; calcd. for one amino nitrogen atom per molecular weight of 816; N, 1.68%. Thus considerably less than one molecule of ammonia is taken up per molecule of polymer, which, in the light of the molecular weight determinations, indicates that the actual molecular weight may be greater than 816, and that possibly dissociation occurs in solution.

METHYL GLYCIDOL AND ITS DIMER

Preparation of Methyl Glycidol

Crotyl alcohol (22 gm.; b.p., 116-119° C.) was treated with a chloroform solution of benzoylhydroperoxide containing 4 gm. of active oxygen for 24 hr. at 0° C. Yield of methyl glycidol, 3 gm. (b.p., 93-97° C./44 mm.).

Methyl glycidol is a mobile liquid with a pleasant odor. The phenyl urethane, crystallized from benzene-ligroin mixture, melted at 66.5° C. Found: C, 63.54, 63.99; H, 6.06, 5.96%. Calcd. for $(\text{C}_{11}\text{H}_{15}\text{O}_3\text{N})_x$: C, 63.77; H, 6.28%.

Polymerization of Methyl Glycidol by Heat

Methyl glycidol (0.5 gm.) on heating in a sealed tube at 105° C. for 22 days was converted into a viscous yellow liquid.

A small amount of this product was added to an equal weight of phenyl isocyanate and allowed to stand for three days when the mixture had changed to a clear yellow solid. By dissolving in benzene, precipitating with ligroin

and then again re-precipitating from ether solution, the diphenyl urethane derivative was obtained as a cream-colored powder; m.p., 78-80° C. This material decolorized dilute potassium permanganate solution, and, when dissolved in toluene, showed no action towards sodium. Analysis indicated that the substance was a nearly pure diphenyl urethane of dimeric methyl glycidol. Found: N, 6.22, 6.30%. Calcd. for monophenyl urethane derivative of dimeric methyl glycidol: N, 4.74%; for diphenyl derivative; N, 6.98%.

OXIDATION OF DIPROPENYL GLYCOL WITH BENZOYLHYDROPEROXIDE

Dipropenyl glycol (18 gm.; b.p., 105-109° C./5-6 mm.) was added to a chloroform solution of benzoylhydroperoxide containing 4.0 gm. of active oxygen. After standing two days at 0° C. the theoretical amount of oxygen had been absorbed.

Fractionation of the reaction mixture under reduced pressure yielded several small liquid fractions, one of which, boiling at 125-128° C./4 mm. (n_D^{25} , 1.4648), had a molecular weight of 190. Calcd. for 2 : 3, 6 : 7-di-epoxy-4, 5-dihydroxy octane, 174. On raising the temperature to 148° C./4 mm. most of the product was left in the distilling flask as a solid resin (10 gm.). This was purified by dissolving in 40 cc. of benzene and then precipitating by the addition of 75 cc. of ligroin, the operation being repeated three times. The final product possessed a yellowish brown color, and presumably still contained some impurity as is indicated also by the analysis. Found: C, 55.43, 55.67; H, 7.68, 7.62%. Calcd. for $(C_8H_{14}O_4)_2$: C, 55.17; H, 8.05%. Mol. wt. $(C_2H_4Br_2)$, 431; calcd. for $(C_8H_{14}O_4)_3$, 422.

PHENYL GLYCIDOL ACETATE

Preparation and Properties

Cinnamyl acetate was prepared by acetylating 50 gm. of cinnamyl alcohol with 76 gm. of acetic anhydride and 10 gm. of sodium acetate. Colorless oily liquid; b.p., 135-138° C./9 mm. n_D^{22} , 1.5390. Yield, 86%.

The acetate (53 gm.) was added to a chloroform solution of benzoylhydroperoxide containing 6.0 gm. of active oxygen, and the mixture allowed to stand for three days at 0° C. The reaction product on fractionation yielded phenyl glycidol acetate as a heavy oil; b.p., 129-132° C./3 mm. n_D^{22} , 1.5208. Yield, 70%. It decolorized cold dilute potassium permanganate solution immediately. Found: C, 68.59; H, 6.58%. Calcd. for $C_{11}H_{12}O_3$: C, 68.75; H, 6.35%. Mol. wt. $(C_2H_4Br_2)$, 190; (benzene), 183; calcd., 192.

Behavior on Heating

Experiment I. Phenyl glycidol acetate (5 gm.) when heated for six days at 145-165° C. at atmospheric pressure was converted into a very viscous substance.

Experiment II. Phenyl glycidol acetate (10 gm.) was heated in a sealed tube for four weeks at 140° C. The resulting product was a thick viscous liquid.

The products from Experiments I and II were mixed together, then dissolved in benzene, and ligroin added. The solid (A) separating out was filtered off, the solvent removed from the mother liquor and the residual oil (B) fractionated.

Fractionation of (B)

- Fraction (1) 100-105° C./0.13 mm. Wt., 0.5 gm. n_D^{25} , 1.5141.
(2) 90-94° C./0.12 mm. Wt., 0.5 gm. $n_D^{24.5}$, 1.5199.
(3) 126-127° C./0.15 mm. Wt., 2.0 gm. $n_D^{24.5}$, 1.5144.
(4) 140-145° C./0.10 mm. Wt., 2.5 gm. $n_D^{24.5}$, 1.5377.

On analysis only fraction (4) was found to correspond empirically with phenyl glycidol acetate. Found: Fraction (4): C, 68.50, 68.43; H, 6.57, 6.66%. Calcd. for $(C_{11}H_{12}O_3)_2$: C, 68.75; H, 6.35%. Mol. wt. $(C_2H_4Br_2)$; 256: (benzene) 285; calcd. for $(C_{11}H_{12}O_3)_2$, 384.

The product (A) precipitated from the benzene-ligroin mixture (1 : 1) was a solid red resin (9 gm.). Found: C, 68.48; H, 6.51%. Calcd. for $(C_{11}H_{12}O_3)_2$: C, 68.75; H, 6.35%. Mol. wt. $(C_2H_4Br_2)$, 623. This material is apparently a polymer with an average molecular weight corresponding to three to four moles of phenyl glycidol acetate.

PHENYL GLYCIDOL METHYL ETHER

Preparation and Properties

Cinnamyl alcohol (40 gm.) was methylated with 130 gm. of methyl iodide and 180 gm. of silver oxide. The methyl ether was obtained as a liquid having a very pleasant odor. Yield, 87%; b.p., 92° C./5 mm. n_D^{20} , 1.5443. Mol. wt. $(C_2H_4Br_2)$, 147; calcd., 148.

Treatment of 36.1 gm. of methyl cinnamyl ether with benzoylhydroperoxide yielded phenyl glycidol methyl ether; b.p., 130-137° C./21-23 mm. n_D^{20} , 1.5170. Found: CH_3O , 18.83%. Calcd. for $(C_{10}H_{12}O_2)$, 18.90%. Mol. wt. $(C_2H_4Br_2)$, 163, 166; calcd., 164. Yield, 34 gm.

Behavior on Heating

Portions of phenyl glycidol methyl ether were heated in sealed tubes as follows: (a) 9.6 gm. with a trace of moist potassium hydroxide; (b) 4 gm. with a trace of moist zinc chloride; and (c) 4 gm. without a catalyst. The tubes were heated at 140-150° C. for 11 weeks, a thick reddish liquid being produced in each case. In (b) a small amount of water separated out.

The products from experiments (a) and (c) were mixed and fractionated:

- Fraction (1) 94-96° C./3 mm. (wt. 4 gm.) n_D^{25} , 1.5100, colorless oily liquid.
(2) 100-105° C./0.05-0.06 mm. (wt. 1.0 gm.) $n_D^{24.5}$, 1.5617, heavy yellow liquid.
(3) 125-130° C./0.03 mm. (wt. 1.5 gm.) $n_D^{24.5}$, 1.5858, viscous yellow liquid.

The residual product was dissolved in benzene and a portion precipitated by the addition of ligroin. This treatment was repeated from benzene and

ether solutions, respectively, yielding 1.0 gm. of a light brown powder (Fraction 4). Evaporation of the solvent from the mother liquor yielded 3.0 gm. of a viscous red liquid (Fraction 5).

Found: Fraction (1) C, 69.96, 69.88; H, 7.65, 7.76%; CH_3O , 20.52, 20.31%. Calcd. for $(\text{C}_{10}\text{H}_{12}\text{O}_2)_2$: C, 73.17; H, 7.32; CH_3O , 18.90%. Fraction (2) CH_3O , 14.48%; Fraction (3) CH_3O , 12.61%; Fraction (4) CH_3O , 6.73%; Fraction (5) CH_3O , 10.91%.

Under the conditions indicated, phenyl glycidol methyl ether does not polymerize, but apparently undergoes other complex changes with formation of viscous and solid resins.

DIMERIC 2-METHOXY ETHYL GLYCOLOSIDE

Dimeric 2-methoxy ethyl glycoloside was prepared by the methylation of the sodium salt of hydroxy ethyl vinyl ether (22). Bromoethylidene glycol (75 gm.) and 150 cc. of pure dry ether were placed in a 500-cc. three-necked flask fitted with a mercury-seal stirrer and a reflux condenser with calcium chloride tube, and 21.5 gm. of sodium, cut in small pieces, introduced in one addition. The mixture was vigorously stirred and, after the reaction had subsided, the contents was heated at a temperature of 70-80° C. for seven hours. The bulk of the ether was then removed by distillation. Methyl iodide (77 gm.) was added and the mixture gently heated on a water bath with stirring for 16 hr. Water was then slowly added until the salts had dissolved. The aqueous solution was extracted twice with ether, the combined ether solutions washed with small amounts of water and dried over anhydrous potassium carbonate.

On fractionation 26.1 gm. of methoxy ethyl vinyl ether was obtained as a mobile, colorless liquid having a rather penetrating musty odor; b.p., 107-110° C./760 mm. n_D^{20} , 1.4130. Mol. wt. $(\text{C}_2\text{H}_4\text{Br}_2)$, 105; calcd. for $\text{C}_8\text{H}_{10}\text{O}_2$, 102.

Oxidation of Methoxy Ethyl Vinyl Ether

The ether (23.6 gm.) was oxidized with benzoylhydroperoxide containing 3.8 gm. of active oxygen. Fractionation of the reaction product yielded several small, indefinite fractions, and a large pale yellow liquid fraction (3.5 gm.). The latter, on redistillation, gave a colorless oil; b.p., 118-119° C./0.1 mm. n_D^{22} , 1.4674. Found: CH_3O , 26.40, 26.34%. Calcd. for $(\text{C}_8\text{H}_{10}\text{O}_3)_2$, 26.27%. Mol. wt. $(\text{C}_2\text{H}_4\text{Br}_2)$, 242; calcd, 236. The product was therefore a dimer of 2-methoxy ethyl glycoloside.

Addition of ammonia. Treatment with alcoholic ammonia gave a reddish oil. Found: N, 0.82, 0.95%. Calcd. for $(\text{C}_8\text{H}_{10}\text{O}_3)_2 + 2\text{NH}_3$, N, 10.37%; for $(\text{C}_8\text{H}_{10}\text{O}_3)_2 + \text{NH}_3$, N, 5.53%.

This result indicates that the dimer is not an associated compound containing two ethylene oxide rings, but more probably a stable dioxane

structure formed by the union of the two oxide rings. The small amount of ammonia reacting may indicate either an equilibrium with an ethylene oxide form, or be due to slight ring fission of the dioxane structure.

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THE ALKALOIDS OF FUMARACEOUS PLANTS

IV. *Adlumia fungosa*, GREENE

BY RICHARD H. F. MANSKE

Abstract

An investigation of the alkaloids of *Adlumia fungosa* has confirmed the presence of *protopine*. The alkaloid previously reported as melting at 176-177° C. proved to be *bicuculline*. Two new alkaloids, *adlumine*, $C_{31}H_{41}O_6N$, and *adlumidine*, $C_{19}H_{17}O_6N$ (?) have been isolated. These names have been used because it is felt that the alkaloids described by Schlotterbeck under the same names were either impure or improperly analyzed. The investigation is being continued.

Schlotterbeck and Watkins (5) and Schlotterbeck (4) have recorded the isolation of five alkaloids from *Adlumia fungosa*, Greene (*A. cirrhosa* Raf.), two of which were identified as *protopine* and α -*allocryptopine* respectively. The other three bases were regarded as new. One, *adlumine*, was stated to possess the empirical formula, $C_{39}H_{41}O_{12}N$, and its melting point was given as 188° C. (? corr.). Another was named *adlumidine* and was regarded as being represented by $C_{30}H_{29}O_9N$; m.p., 234° C. (? corr.). The fifth was not named, and on account of the small quantity isolated, its composition was not determined, but it was stated to melt at 176-177° C. (? corr.).

Adlumia fungosa (N. O. Fumariaceae) is a handsome climbing biennial which under favorable conditions attains a length of 25 to 30 ft. It is widely distributed throughout eastern North America, but in general it is found in any one locality in only small amounts. It was therefore a matter of good fortune to find it in sufficient quantity for a chemical investigation, and in continuation of a program of research outlined previously (1) its collection and chemical examination were undertaken.

The presence of *protopine* was readily confirmed, but if α -*allocryptopine* is present at all in the available specimen, the amount is exceedingly minute, since the total uncrystallized mixture of bases from the mother liquors amounted to only a very small fraction of the total bases. The alkaloid melting at 176-177° C. was readily obtained and proved to be identical with *bicuculline* (2, 3).

In regard to Schlotterbeck's *adlumine* and *adlumidine* the situation is complicated by the unusual compositions ascribed by him to these bases. It is certain that no alkaloids of such composition were present in tractable amounts in the material under investigation. At the same time, however, an alkaloid melting at 235° C.* has now been obtained. Analyses yield figures which are in substantial agreement with $C_{19}H_{17}O_6N$ or with $C_{19}H_{15}O_6N$. Since the substance appears to be the same as that to which Schlotterbeck assigned the name *adlumidine* this name will be retained for it.

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Contribution from the National Research Laboratories, Ottawa, Canada.

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*All melting points are corrected.

Further, it is proposed to transfer (or retain) the name adlumine to an alkaloid melting at 180° C. which was obtained in the course of the present investigation, the empirical formula of which is $C_{21}H_{21}O_6N$. It is probable that Schlotterbeck's adlumine was the higher melting form of bicuculline (196° C.) contaminated with some adlumine. Such mixtures have been encountered in the present work and their separation by fractional crystallization was accomplished only in part.

During the various investigations of Fumaraceous plants which the author has completed in whole or in part, a unified procedure has been developed which appears to have certain advantages over the various methods hitherto described. Investigations in progress, dealing with *Dicentra eximia*, *Corydalis sempervirens* and *C. aurea* have shown the usefulness of the method, and since any modifications necessitated by special conditions are of a minor nature it seems desirable to describe the method in full in this communication. In subsequent publications it is proposed to refer merely to the specific labels which will be given to each fraction. It is thus hoped to avoid the lengthy repetition which would otherwise be necessary in each case.

Experimental

Preparation of the Extracts

The ground plant material is thoroughly extracted with methanol in a Soxhlet apparatus and the solvent largely distilled (Extract-E). Much hot water is added and the mixture rendered acidic to Congo red with hydrochloric acid. The remainder of the methanol is boiled off, preferably under reduced pressure and the mixture cooled. In general the resinous and fatty products are difficult to remove by filtration, but if the mixture is allowed to remain in an ice chest for several days or weeks the aqueous solution frequently becomes perfectly clear and can be separated from the residue (R) by decantation. The latter is again treated with very dilute boiling hydrochloric acid, and cooled as before and the two decantates combined (Aqueous Extracts-S).

In more difficult cases it may be necessary to add chloroform to the aqueous mixture and filter through a layer of charcoal, but this is not recommended if it can be avoided.

The aqueous solution (S) is treated with a little charcoal in the cold, filtered and thoroughly exhausted with chloroform. The combined extract (C) is evaporated to a convenient volume and clarified by filtration with a little charcoal. In general it is inadvisable to attempt to obtain pure products directly from this extract. The aqueous solution from which chloroform no longer removes appreciable amounts of extracts is filtered from a small amount of amorphous deposit (D) insoluble in both media (Filtrate-SC).

Examination of the Chloroform Extract (C)

The solvent is largely removed and the residue boiled with dilute hydrochloric acid. Separation of the insoluble residue (RC) may be effected either by filtration, decantation or ether extraction, depending largely upon its

properties. In any case the aqueous solution (SR) is exhausted with ether and yields the extract (LC) which, depending upon its nature, may or may not be combined with (RC). The ether is expelled from the aqueous solution and the latter basified with excess potassium hydroxide. When the precipitate (BC) has become granular it is filtered off and washed with water and the filtrate (FC) exhausted with ether (Extract-EC). The residual aqueous solution (CES) is saturated with carbon dioxide and the precipitated base (BCE) filtered off. The filtrate is again exhausted with ether and yields the extract (EEC).

Examination of the Filtrate (SC)

An excess of ammonia is added to this solution and the mixture thoroughly extracted with chloroform. When large quantities of extract are being manipulated it is frequently desirable to allow the basified solution to remain in contact with chloroform in a cold place for several days to allow the complete separation of ammonium magnesium phosphate and other inorganic substances. The mixture is then filtered and the insoluble residue thoroughly washed with chloroform. Separation of the chloroform layer is thus greatly facilitated in the subsequent extractions.

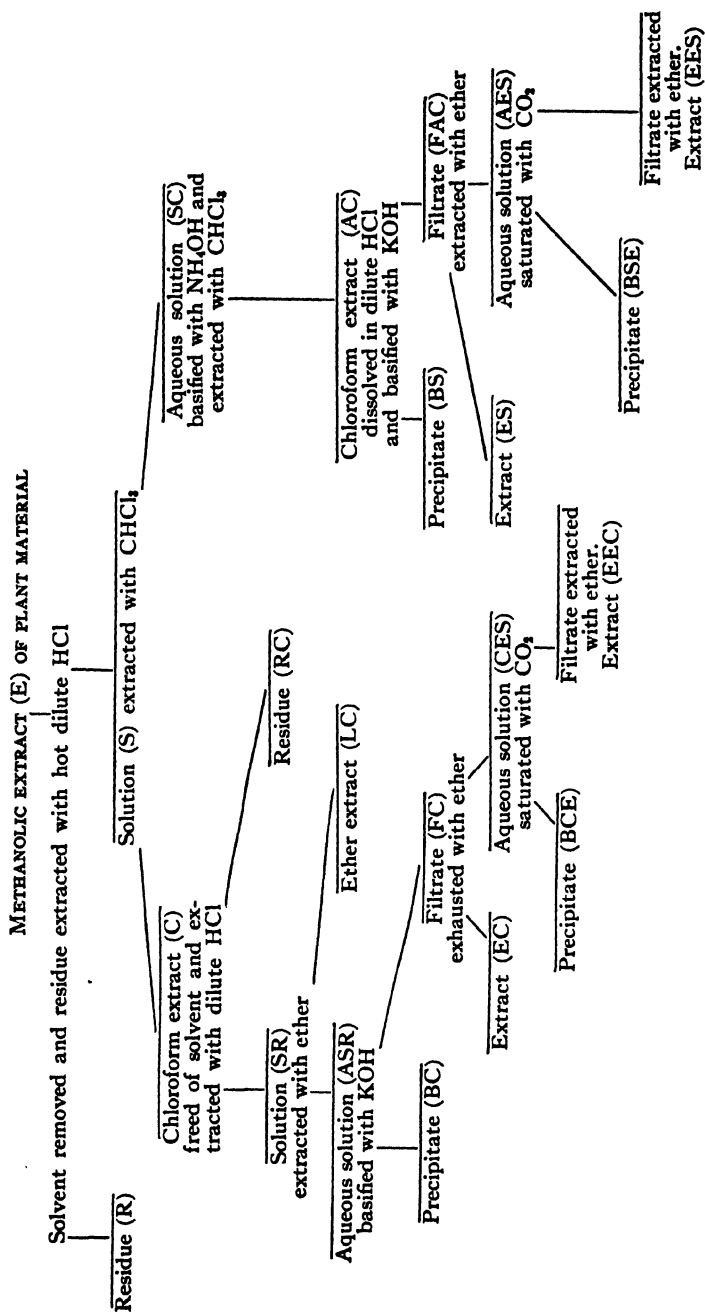
The combined chloroform extract (AC) is evaporated to a convenient volume and filtered through a layer of charcoal to remove some suspended matter. In general it is difficult to obtain crystalline products directly from this solution and for the purpose of further separation and purification the solvent is removed and the residue dissolved in hot dilute hydrochloric acid. A small amount of insoluble resin, which is generally present here, is removed with the aid of charcoal. The cooled filtrate is basified with excess potassium hydroxide and the precipitate (BS) filtered off after it has become granular or coherent and the filtrate (FAC) exhausted with ether (Extract-FS). The residual aqueous solution (AES) is saturated with carbon dioxide and the precipitated base (BSE) filtered off. The filtrate is again extracted with ether and yields the extract (EES).

It is to be pointed out that the separations above outlined while not strictly quantitative, nevertheless effect a fractionation which greatly facilitates the subsequent isolation and crystallization of pure individuals. The further treatment of each fraction is obviously dependent upon its properties and may be different for different plants.

The included flow sheet is included in the interests of clarity.

The author's experience does not enable him to allocate all or most of the known alkaloids of Fumraceous and Papaveraceous plants in this scheme, but it may be of interest to review briefly where a number of them are found.

Dicentrine (and probably also glaucine) is found in the non-phenolic fraction (BC) of the chloroform extract (C). Corydine and isocorydine are removed from the alkaline solution by means of ether (EC). Bicuculline is precipitated only by means of carbon dioxide (BCE) and (EEC).



The non-phenolic alkaloids (BS) contain the protopine and cryptopine, while bulbocapnine is not removable from the alkaline solution by means of ether. It is therefore found in the fraction (BSE).

Adlumia fungosa

There was available 11 kilos of dried plant of which 255 gm. was the underground portion. Each portion was examined separately, but since all the products to be described subsequently were found in both parts only the examination of the stems and leaves need be recorded.

Isolation of Fumaric Acid

The insoluble residue (R) was extracted with several successive portions of hot water and the combined extracts clarified with charcoal. On cooling, a copious deposit of fumaric acid was obtained. By means of several recrystallizations which included concentration of the mother liquors there was obtained 70 gm. of pure fumaric acid melting at 295° C. (286° C. uncorr.). Comparison with an authentic specimen of fumaric acid failed to disclose any differences.

Isolation of Bicuculline and Adlumine

The experience gained while working with the alkaloids from the roots showed that the chloroform extract (C) contained only these two alkaloids in tractable amounts and the same proved to be true in the case of the aerial portion.

The acid solution of the bases (ASR) was basified with ammonia and extracted with chloroform. The combined extracts were evaporated to a syrup and the latter repeatedly extracted with much ether until only an insignificant amorphous residue remained. Evaporation of the extract to a small volume yielded a thin syrup which gradually crystallized in contact with methanol. Examination with a lens disclosed the presence of two products which were separated by a long series of fractional crystallizations from a variety of solvents,—methanol, ethyl acetate, acetone, etc. From the more soluble fractions a base (m.p., 180° C.) was obtained. It proved to be identical with adlumine, the greater portion of which was present in the mixture of bases not removed by chloroform from acid solution (see above).

The less soluble fractions yielded a pale yellow base, the melting point of which was raised only slowly from 192–194 to 194–196° C.

For the purpose of further purification it was dissolved in chloroform-methanol, rendered just acid with concentrated hydrochloric acid, and the solution repeatedly evaporated with chloroform to remove other solvents. A slight turbidity was removed with the aid of charcoal and the colorless filtrate evaporated to a small volume. On cooling, colorless plates of a hydrochloride rapidly separated. Ethyl acetate was added to facilitate manipulation

and filtration. The hydrochloride as thus obtained melted at 259° C. and no depression was observed when it was admixed with a specimen of bicuculline hydrochloride from *Dicentra cucullaria* (3). Yield, 4 gm.

The base regenerated from the hydrochloride was recrystallized from chloroform-methanol and melted at 196° C. When however the supersaturated solution was seeded with a crystal of bicuculline melting at 177° C. the same form was obtained, and no depression in melting point was observed when the two were mixed. Oxidation with dilute nitric acid yielded hydrastine.

Isolation of Adlumidine and Adlumine

The chloroform extract (AC) was evaporated to a small volume, filtered with the aid of charcoal and evaporated to a thin syrup. In the course of several days a crop of colorless sparingly soluble crystals had separated. Examination under a lens and subsequent recrystallization proved this alkaloid to be homogeneous.

It was filtered off, washed first with chloroform-methanol and then with methanol. It was dissolved in a large volume of chloroform, in which it is sparingly soluble, an equal volume of hot methanol added and a slight turbidity removed by filtration with the aid of charcoal. The colorless filtrate was rapidly evaporated. While still hot, colorless fine stout prisms separated. After cooling the base was filtered off, washed first with hot methanol, then with a little chloroform, and again with methanol. After drying, the *adlumidine* as thus obtained melted sharply at 235° C. to an orange-colored liquid without visible decomposition. Recrystallization changed neither the appearance nor the melting point. Yield, 5 gm. Calcd. for $C_{19}H_{18}O_6N$; C, 64.59; H, 4.25; N, 3.97%. Mol. wt., 353. Calcd. for $C_{19}H_{17}O_6N$; C, 64.23; H, 4.79; N, 3.94%. Found: C, 64.98, 65.14; H, 4.79, 4.75; N, 3.86, 3.92%. Mol. wt., 297, 308 (Rast). Methoxyl, negative.

The filtrate from the crude adlumidine by a fortunate chance was allowed to remain overnight, during which time large almost colorless rhombic plates separated. This product was filtered off, washed with cold methanol and recrystallized from chloroform-methanol. Fine rhombic plates melting sharply at 180° C. were thus obtained. Yield, 6 gm. This proved to be identical with the *adlumine* previously isolated from the chloroform extract (C). Calcd. for $C_{21}H_{21}O_6N$; C, 65.80; H, 5.48; N, 3.66; 2 OMe, 16.15%. Found: C, 66.02; H, 5.53; N, 3.88; OMe, 15.71% (mean of duplicates).

Isolation of Protopine

The mother liquor from the adlumine was freed of organic solvents and the residue dissolved in dilute hydrochloric acid. A slight turbidity was removed with the aid of charcoal, and the cooled filtrate treated with excess potassium hydroxide. After several days the granular base was filtered off, washed with water and dried. It was recrystallized by solution in a mixture

of chloroform and methanol and evaporating the clarified filtrate (charcoal) to a small volume. When a crystal of protopine was added to the hot solution a copious crop of this alkaloid rapidly crystallized out. Yield, 7 gm.

There are two crystal forms of protopine analogous to the two forms of allocryptopine. The more common, but less stable, form consists of warty aggregates of excessively minute needles, which melt at 205-206° C. The more stable form melts at 211° C. when pure and consists of large stout highly refracting prisms with many faces developed. It is much less soluble in chloroform and a mixture of the two may be easily separated by means of this solvent. when, as frequently happens, they crystallize side by side. When slowly heated a mixture of the two forms melts at 210-211° C.

The specimen of protopine obtained from *Adlumia fungosa* melted at 211° C. either alone or admixed with a specimen prepared for purposes of comparison from *Dicentra spectabilis*. Calcd. for $C_{20}H_{19}O_5N$; C, 67.99; H, 5.38; N, 3.97%. Found: C, 67.81; H, 5.45; N, 4.02% (mean of duplicates). The alkaline filtrate from the protopine was extracted with ether which removed a little protopine. It was then saturated with carbon dioxide and the whole thoroughly extracted again with ether. There was thus obtained about four grams of an alkaloid which proved to be adlumine.

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STUDY OF A FILTER-PASSING STRAIN OF A STREPTOCOCCUS ISOLATED FROM A CASE OF BOVINE MASTITIS¹

BY FRANCES H. PRISSICK²

Abstract

A strain of green streptococcus, No. 403, obtained from a case of bovine mastitis and similar to that usually associated with chronic mastitis in cattle, has under stated conditions been passed through Berkefeld N and Chamberland L 3, L 5 filters. It has been recovered from the filtrates and proved to be identical in its biological characteristics with the original unfiltered strain No. 403.

Filtrates of this organism grown in K medium have given the largest number of recoveries, these being 18 out of 20, or 90%. Filtrates of growth in nutrient broth were recovered in 1 out of 20 filtrations, or 5%.

Observations on the cultural and morphological appearance of the organism during the stages of its recovery are made. The interpretation of these experiments in the light of Zinsser's postulates is discussed.

During the past few years a large school of bacteriologists has grown up, which has substituted the theory of a monomorphic life of the bacterium for that of a metamorphic one. The conclusions reached by investigators from their experiments are well reviewed by Evans (1) and Hadley, Delves and Klimek (4). Only those results which are directly pertinent to the subject of this paper will be mentioned in any detail.

In 1926, Ramsine (12) reported the discovery of a growth in culture media which were being incubated for sterility during the process of making Dick toxin. This growth was first observed after an incubation period of three or four days in dextrose bouillon. It appeared as a light sediment or flocculence, which staining showed to consist of Gram negative mycelium, containing Gram positive granules. Further culture and incubation gave rise to forms which resembled streptococci in morphological appearance, and which later developed into definite cocci in chains.

Hauduroy (5) claimed to have filtered various kinds of bacteria through new filter candles, and to have recovered these organisms in their recognized form, after numerous passages on lactose litmus agar. Later, Hauduroy and Lesbre (6) described a technique for the cultivation of hemolytic streptococci from the filtrates of their growth in peptone water. The type of filter candle used in these filtrations is not mentioned. The organism appearing in these cultures corresponded closely in its morphology to that noted by Ramsine (12). Out of 26 strains of streptococci filtered, 10 produced filter-passing forms. This work was later confirmed by Urbain (15).

Dealing principally with the members of the dysentery group, Hadley, Delves and Klimek (4) were able to dissociate this bacillus into R, S, and G colonies by means of incubation in bouillon containing pancreatin or lithium chloride; the G type proved to be filter-passing.

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Contribution from the Animal Diseases Research Institute, Hull, P.Q. This paper is a preliminary report.

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In 1931, Kendall (10) advocated the use of a new medium as a means of dissociating bacteria and as a producer of filter-passing forms. This medium is made from intestine, preferably that of swine. Other tissue such as heart, brain, spleen or kidney of various animals has been used, but has proved less satisfactory than that of hog intestine. The finished medium is said to contain protein of a naturalness closely approximating that of the animal body, and to be almost free from protein degradation products. Kendall (9) has claimed to have passed several types of streptococcus, *B. typhosus*, *H. influenzae*, *Staphylococcus aureus*, and Noguchi's *Leptospira icteroides* through Berkefeld N filters, after growth has taken place in his K medium. He also suggests its use in the cultivation of the filterable viruses.

At the time of writing this paper, very few workers have come forward with results obtained in the use of this medium. Among these are Hoffstadt and Youmans (7) who have used K medium as a means of dissociating a strain of *Staphylococcus aureus* into R, S, and G type colonies; it was effective in culturing the G forms.

Rivers (13) found K medium unsuitable for the cultivation of vaccinia virus.

Zinsser (17) has stressed the necessity for postulates in dealing with the problem of filterability and a bacterial life cycle. The need for such postulates is great, because of the confusion and misunderstanding existing amongst workers on this subject, due chiefly to the propounding of theories unaccompanied by adequate proof.

The bearing of these postulates on this work will be discussed at the conclusion of the following preliminary report of experiments with K medium upon the possible filter-passing properties of a strain of green streptococcus.

STREPTOCOCCUS. (STRAIN NO. 403)

Viridans Group, Type α

Source

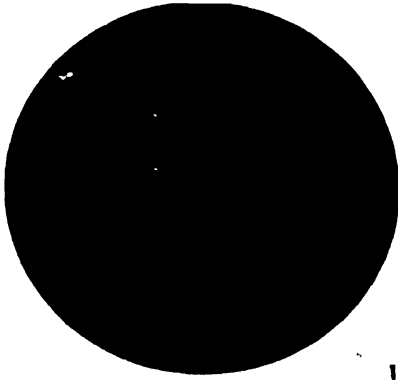
The streptococcus used in these filtration experiments was isolated by Dr. Rosell of Oka from the milk of a cow with chronic mastitis, and is of the type usually associated with that disease.

Colony

This is fairly small, measuring from 0.25 to 0.5 mm. in diameter, flat, opaque, smooth with edges entire, and growing well on chocolate agar at 37° C., where a yellowish green ring of coloration is produced, in ratio to the colony 2 : 1 (Figs. 1 and 2).

Morphology

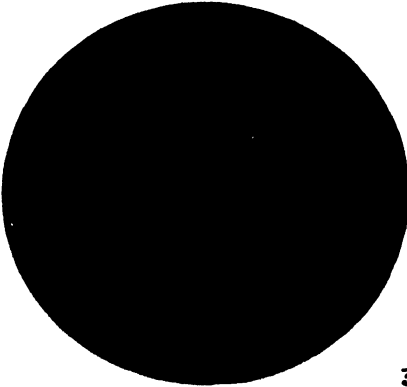
When grown on chocolate agar at 37° C. for 24 hr., the streptococcus measures from 0.8 μ to 0.9 μ in diameter, taking a deep Gram positive stain, and growing as single rather oval cocci, paired or in short chains. In lactose broth, chains of medium length are produced. No capsule has been demonstrated.



1



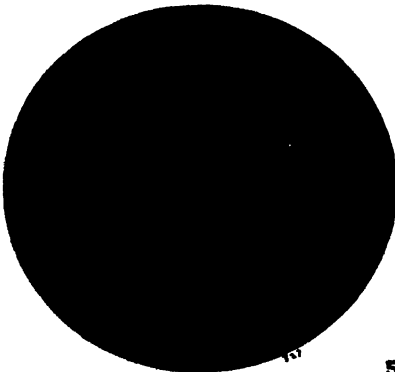
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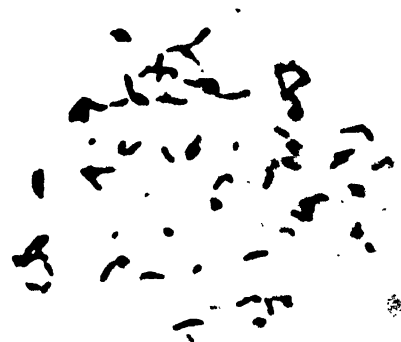
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6

FIG. 1. Colonies of *Streptococcus* 403 on chocolate agar, 24 hr. at 37° C. X 4. FIG. 2. *Streptococcus* from the same type of colony; Gram's stain. X 1300. FIG. 3. Type A colony, "Stage 1". X 15. FIGS. 4 and 5. Type of organisms found in "Stage 1" colonies. (FIG. 4. Methylene blue. X 1300. FIG. 5. Gram's stain. X 1300.) FIG. 6. Type of organisms found in older "Stage 1" colonies; methylene blue. X 1300.

Biochemical

Sugar reactions were read after five days' incubation at 37° C. in Hiss' serum water, made with 1% of each sugar and 1% of Andrade's indicator.

Lactose	Mannite	Salicin
Acid	—	—
Coagulation (slight)	—	—

Pathogenicity

Rabbits remained unaffected by a dose of strain No. 403 grown 24 hr. on blood agar, re-suspended in 15 cc. of normal saline, and from 5 cc. to 10 cc. injected intravenously.

The inoculation of 1 cc. of a 24-hour-old culture on chocolate agar, re-suspended in 10 cc. of normal saline, into the peritoneal cavity of a mouse, leads to no noticeable clinical signs or symptoms.

Technique

Below are given the details pertaining to the preparation of the principal media and the filtration apparatus as used in all the experiments reported.

*K medium***MEDIA**

1. The small intestine of a hog was washed, chopped fine and extracted with four volumes of 95% alcohol for 48 hr. at 37° C. This extraction was repeated twice, making three extractions in all.

2. The alcohol was removed and the tissue extracted once again with two volumes of benzol, until the tissue looked brown and clear.

3. The tissue was dried in warm air, and ground as fine as coffee grounds in a ball grinder or very fine meat chopper.

4. Two per cent of the dried tissue was added to 1000 cc. of Tyrode solution, for which either of the formulas in Table I proved to be satisfactory.

TABLE I

Formula No.	NaCl, gm.	KCl, gm.	CaCl ₂ , gm.	MgCl ₂ , gm.	NaH ₂ PO ₄ , gm.	NaHCO ₃ , gm.	Dextrose, gm.	Aqua dist., cc.
1	8.0	0.2	0.2	0.01	0.05	0.2	0.8	1000
2	7.0	0.2	0.2	0.1	0.05	1.0	1.0	1000

5. The solution was heated in flowing steam for 25 min. and the sediment allowed to settle.

6. The pH was then adjusted with sodium bicarbonate to 7.4–7.5.

7. The supernatant fluid was decanted and filtered through a Berkefeld N filter candle, and retitrated, if found necessary. The medium was then distributed into tubes in 15-cc. and 10-cc. amounts.

8. After sterilization in the autoclave for 20 min. at 15 lb. the tubes were incubated 48 hr. for sterility.

The final pH was 7.3 to 7.4.

Agar

In culturing the filtrate, two agar bases were used, to the first of which was added 3% of fresh sterile ox-serum, and to the second the same percentage of fresh defibrinated ox-blood. The agar was melted in the autoclave and cooled to 45° C., the sterile serum or blood mixed thoroughly with each, and petri dishes poured. The plates were incubated 48 hr. at 37° C. for sterility. Both kinds of agar were used in all transfers from the cultures of the filtrates of growth in K medium and nutrient bouillon. For the sake of convenience, these have been termed respectively agar No. 1 and No. 2.

Agar base No. 1. To 1000 cc. of beef infusion are added: 0.5% of sodium chloride, 2.0% of proteose peptone (Difco), 1.5% of dried intestine, 0.8% of dextrose (c.p.). The mixture is heated for 45 min. at 70° C. and titrated with sodium bicarbonate to pH 7.5. Agar (3%) is added to this and melted in the autoclave. The fluid is titrated and distributed into flasks which are sterilized for 25 min. at 15 lb. and then incubated two days for sterility. The final pH should be 7.4.

Agar base No. 2. This medium is a very slightly modified form of Gray's broth (3) for culturing streptococci.

Fresh beef heart, freed from fat and fibre, is ground in a chopper and infused in the ice box overnight, in the proportion of 500 gm. of meat to 500 cc. of tap water.

In the morning this is heated to 20–25° C. and strained through a single layer of flannel. The filtrate is boiled for one hour, filtered through filter paper and made up to volume. The following are added: 1.5% of proteose peptone (Difco), 0.5% of sodium chloride, 1.0% of lactose (c.p.), and 1.0% of gelatin. This is placed in flowing steam for one hour, filtered through filter paper and titrated to pH 7.8–7.9. Agar (2.5%) is added and the whole is steamed until it melts, after which it is distributed into sterile flasks and autoclaved for 30 min. at 10 lb. These are incubated 48 hr. at 37° C. for contamination.

The chocolate agar used in the study of some of the subcultures is that of Crowe.

Apparatus

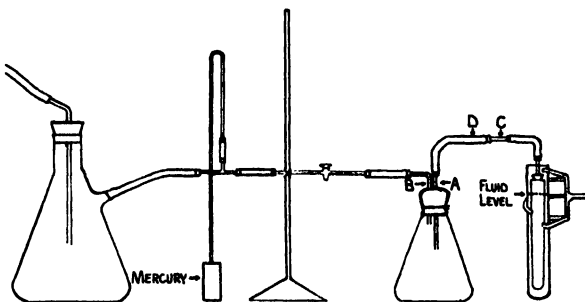
The accompanying illustration shows the set-up of the filtration apparatus.

The filter flask is plugged with a rubber cork, through which run glass tubes, *A* and *B*, and the cork and neck of the flask covered with tin foil. One flask with rubber tube, *D*, is attached to *A*, and the end wrapped in tin foil. Tube *B* is plugged with absorbent cotton. The whole flask with tubing attached is wrapped in paper and sterilized in the autoclave.

The filter candle is fitted with a rubber cork through which is passed narrow glass tubing. One end of this reaches down to the bottom of the candle and the other is fitted tightly with rubber tubing, which is joined to a short glass tube, C. The joints are fastened with wire. Tube C is plugged with absorbent cotton and wrapped in tin foil. The candle and its fittings are then wrapped in paper and sterilized in the autoclave.

The flask is unwrapped and the tin foil wrappings covered with boiling paraffin.

Half of the glazed portion of the candle, the cork, and part of the rubber tubing attached, are also covered with several layers of boiling paraffin. Tube B is connected with the suction pump. Taking sterile precautions, tube D is quickly joined to C. The filter candle is inserted into the fluid to be filtered, and pressure applied slowly.



Filtration apparatus.

Filter Candles

Filtration took place through only new Berkefeld N filters, and through both new and used Chamberland L 3 and L 5 candles.

The Chamberland candles were cleaned by a method recommended by Dr. E. G. D. Murray of McGill University, Montreal. This method has proved to be most satisfactory, as it enables the filters to be used again many times without the clogging of pores and loss of speed.

This method of cleaning is as follows. Contaminated candles are washed through by gravity with about 200 cc. of distilled water and then soaked overnight or longer in 10% hydrochloric acid. They are then washed by running through by gravity not less than 500 cc. of distilled water.* The candles are dried in the air or incubator at 37° C. and soaked in a 10% solution of ammonium nitrate, for a sufficient length of time to saturate the pores. After being dried thoroughly in the air or incubator they are burned at a dull red in a furnace, cooled slowly, washed by gravity using distilled water, and then dried in the incubator and wrapped in grease-proof paper. The unglazed portion of the candles should not be touched with the hands after soaking in hydrochloric acid, as passage through the filter may be facilitated by the presence of grease or oil (8).

The candle was then fitted with rubber cork and glass tubing as described in the diagram of the apparatus for filtering, and autoclaved for one-half hour at 15 lb. One set of each grade of filter employed was reserved solely for use in these filtrations, so that used candles had only been in contact with the control organisms which will be mentioned, and Streptococcus No. 403.

*A large glass container holding 1500 cc. of distilled water is used. To the neck of this is attached a 2-in. piece of rubber tubing, which is fitted onto the candle. The whole head is not more than 4 in. long.

Experimental

Twenty filtration experiments on the possible filter-passing properties of this strain of *Streptococcus* No. 403 have been completed, the procedure following three main steps.

1. Incubation in K medium with control broth cultures.
2. Filtration through Berkefeld N or Chamberland L 3, L 5 candles.
3. Culture of the filtrates, with the purpose of recovering, if filter-passing, an organism with all the biological characteristics of the original strain No. 403.

All these experiments have been carefully charted, and as far as possible, all details considered essential to the filtration process recorded (11). One of these protocols is given as an example (see Table II).

I. Growth of the Cultures Prior to Filtration

The streptococcus was seeded into tubes containing 15 cc. of K medium and nutrient broth respectively, and incubated at 30° C. Uninoculated tubes were incubated with these as controls. The length of time for incubating the cultures depends upon the rapidity with which the organism forms a type of growth in K medium, which will be described. This is a fairly heavy even suspension with some precipitation and flocculence. It was found that 65-72 hr. is sufficient incubation time to afford a very definite change in the morphological appearance of most of the streptococci in the medium. On being stained, the growth shows surprisingly few cocci for the density of the suspension. The cocci are Gram positive; the remainder consists of Gram negative fragments and also particles of darker staining matter. These particles, of which only a few have been seen in one microscopic field, are extremely small, being scarcely visible under a magnification of 1800, and are without definite structure as far as has been seen. Dark field examination shows numerous intensely shining bodies, some of which correspond in size to the particles described above. As the uninoculated K medium also shows bright granules, it is difficult to determine what is organic or inorganic material.

Theoretically, if one considers the possibility of there being a filter-passing stage in the life of a bacterium, and that this stage borders on the line between visibility and invisibility, it is not improbable that some of these particles may be connected with an intermediate stage between a filter-passing and non-filter-passing form.

Growth in nutrient broth has the same appearance macroscopically, as that seen in K medium. Microscopically, however, in contrast to the K medium, it shows larger numbers of Gram positive cocci in pairs and chains.

II. Filtration

As Zinsser has pointed out, filtration through ordinary laboratory filters is an uncertain process. So much depends upon the fluid in which the organisms are suspended, as well as on other factors such as the electrical charge of organism and candle, pressure and possible absorption or inactivation during filtration.

TABLE II
FILTRATION EXPERIMENT No. 18. STRAIN—STREPTOCOCCUS No. 403

Medium used	Incubation temp., ° C.	Time, hr.	Growth	Dilution of cultures	Control
"K" medium (made with Tyrode solution No. 2) pH 7.4	30	70	Good growth, even suspension with some flocculence	10 cc. of culture + 20 cc. of freshly sterilized physiological saline	18-hr. culture of <i>B. prodigiosus</i> on nutrient agar, washed off in fresh, sterile, normal saline; four drops of this emulsion added to the diluted culture before applying pressure
Nutrient broth (control) pH 7.4	30	70	Good growth, even suspension with some flocculence	10 cc. of culture + 20 cc. of freshly sterilized physiological saline	18-hr. culture of <i>B. prodigiosus</i> on nutrient agar, washed off in fresh, sterile, normal saline; four drops of this emulsion added to the diluted culture before applying pressure

Filtration temperature	pH of fluid filtered	Filter	Pressure	Time, min.	Amount filtered, cc.	Distribution of filtrate
Room temperature 22° C.	pH 6.8	Used (once) Chamberland L5	4½–5" of mercury	15	Approximately 25–30	0.5 cc. to 10 cc. K medium 0.25 cc. to 10 cc. K medium 0.1 cc. to 10 cc. K medium 0.5 cc. to 10 cc. nutrient broth 0.25 cc. to 10 cc. nutrient broth 0.1 cc. to 10 cc. nutrient broth 1 drop to agars No. 1 and 2
Room temperature 22° C.	pH 6.8	Used (once) Chamberland L5	4½–5" of mercury	14	Approximately 25–30	0.5 cc. to 10 cc. K medium 0.25 cc. to 10 cc. K medium 0.1 cc. to 10 cc. K medium 0.5 cc. to 10 cc. nutrient broth 0.25 cc. to 10 cc. nutrient broth 0.1 cc. to 10 cc. nutrient broth 1 drop to agars No. 1 and 2

Recognizing the influence exerted by any or all of these or other factors, 20 filtrations were performed under the following conditions.

A. Preparation of the cultures. To every 10 cc. of culture to be filtered, 20 cc. of freshly sterilized physiological saline was added, and the pH of the dilution noted. It was thought possible that an acid or alkaline pH might influence the passage or non-passage of the organism through the filter, so the pH was made to vary from 6.0–7.6, as may be seen from the chart which gives a summary of these experiments and their results. In the first nine filtrations, the pH of the K medium culture on filtering was acid or very slightly alkaline, with the pH of the control broth quite definitely alkaline. In the last series, the pH of both K and broth cultures was the same on filtering. The results suggest that the passage or non-passage of the streptococcus through the filter was not influenced by the pH.

B. Process of Filtering

(1) The apparatus was set up as illustrated, with sterile precautions.

(2) As a filter control, an 18-hr. culture on nutrient agar of either *Staphylococcus albus* or *B. prodigiosus* was washed off in freshly sterilized physiological saline, and about four drops of the emulsion added to the culture to be filtered immediately before the pressure was applied.

The *Staphylococcus albus* strain was isolated from milk, and measured from $0.3\ \mu$ to $0.9\ \mu$ in diameter, the average being $0.6\ \mu$.

In later filtrations, the *Staphylococcus* was replaced by a culture of *B. prodigiosus* obtained through the kindness of Dr. T. M. Rivers of the Rockefeller Institute for Medical Research, and which had been used extensively as a filter control. Both organisms grew well on all media employed in these experiments.

(3) Transplants were made from the diluted cultures before filtering in order to be certain of their viability.

(4) Reduced pressure was applied very slowly, increasing until the manometer registered not more than 6 in. of mercury, usually remaining at about 5 in. The time taken to filter approximately 25–30 cc. varied according to the pressure, and grade of the candle, but was never longer than 20 min.

Filtration always took place at room temperature.

III. Culture Technique Following Filtration

The filtrates of the cultures in both K medium and nutrient broth were removed by means of a graduated pipette with a capillary point which reached through tube *A* to the bottom of the flask, and distributed in 0.5-, 0.25- and 0.1-cc. amounts into tubes containing 10 cc. of K medium, and nutrient or dextrose broth. These were incubated at 30° C. In addition, one drop of the filtrate was planted on agar Nos. 1 and 2, and incubated along with the other cultures. In all experiments where petri dishes were used for agar, uninoculated plates were incubated as controls, as were also uninoculated tubes of both K medium and nutrient broth. It may be suggested that

contamination could take place during the processes of putting together and dismantling the filtration apparatus. All possible sterile precautions were taken, and on no occasion was a filtrate found to produce growth other than such as will be described.

Description of Primary Growth

As other investigators have reported, Hauduroy (5, 6), Hadley *et al.* (4) and Ramsine (12), the filtrate growth in its early stage did not resemble that of the original strain of organism, either culturally or morphologically.

In the 20 experiments performed with this strain, No. 403, any growth that was obtained showed first on the agar plates, or in tubes of K medium receiving the filtrate of growth in K medium. Occasionally, and much later, growth occurred in nutrient broth tubes which had been planted with K medium filtrate. Only once has an organism been recovered from a broth filtrate, and that had been incubated in K medium.

The primary growth was difficult to classify. After 48 hr. at the earliest, usually several days however, a very slight sediment could be seen at the bottom of the tube. This sometimes developed into a flocculence. The structure of this sediment and flocculence was indefinite, the main body of it being Gram negative with a few particles which stained faintly Gram positive. Like those found in the growth in K medium before filtering, these particles were also without definite structure, though inclined to be spherical; they might be either organic or inorganic matter. This flocculence has been transferred from tube to tube of K medium, and from the first transplant an organism was recovered. This organism resembled the diphtheroid to be described shortly.

After 48 hr. incubation, or as soon as the sediment appeared in the tube, transplants were made to agar Nos. 1 and 2 and incubated at 30° C. These transplants were made by removing a few drops of fluid containing some of the sediment, by means of a sterile Pasteur pipette, and streaking one drop on agar Nos. 1 and 2. If no growth appeared in 72 hr. the line made by the inoculating loop was scraped and resown on fresh agar, which was incubated again at the same temperature. In addition, fresh agar plates were planted from those tubes of K medium and nutrient broth which had received the K and broth filtrates. This process was repeated until growth in the form of colonies became visible. These took from a few days to almost two weeks to show. No plate culture was ever discarded before two weeks time, unless the agar had become too dry to afford any likelihood of growth.

Recognizing the errors which might be caused by contamination, the following precautions were taken.

When any transplant was being made from tube or petri dish, one of the control plates was exposed to the air for the same length of time, and streaked with the medium from the uninoculated K medium and nutrient

broth tubes. It was not considered necessary to filter these control-tube contents previous to plating out, owing to the fact that the final sterilization of the media was by autoclaving and not by filtration. The only growth ever obtained on these control plates was fungus, and an occasional colony of *Staphylococcus albus*. Nothing suggestive of the growth from cultures of K filtrates appeared. It was thought that the dangers of exposing the plates frequently to the air might be overcome by using Petroff's culture flasks. This was not found to be satisfactory, however, as the water of condensation made it impossible to see the very small colonies on their appearance; though these flasks were used successfully in later transplants, as many as six to eight serial transfers being made in the same flask. Incubation of cultures under reduced oxygen pressure gave no more successful results than did incubation under aerobic conditions.

Development of the Colonies

The first colonies developing on agar from the K filtrate, which had been incubated in K medium or broth, were always similar. For convenience sake, the streptococcus colony as found in the stock No. 403 culture has been called type T colony, and the form recovered in the filtrate growth, type A colony.

The following four main "stages" in the development of the A colony from its first appearance on agar until it regained the T form have been found to occur.

1. The colony was minute, rather glistening, smooth and slightly opaque; and after 48 hr. the centre was seen to be finely granular. These colonies grew with difficulty, it being rare to obtain growth from transplants of them before 48 hr. After a few subcultures, however, they reproduced more rapidly on either agar 1 or 2 at 30° C. (Fig. 3.)

The organism from these colonies was a Gram positive diphtheroid-like rod, slightly curved at times; occasionally coccoid forms could be noted. (Figs. 4, 5, 6). These colonies remained stable in form for several generations, and the organism found in them did not undergo any very noticeable change beyond a slight lengthening. They grew poorly, if at all, in broth.

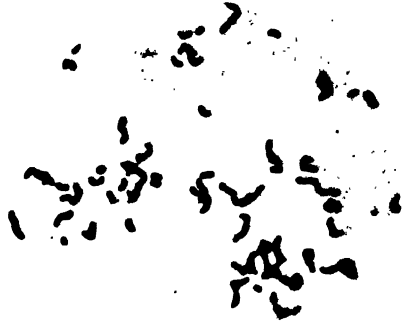
2. The second stage came about gradually. The diameter increased and the colony became more opaque and acquired a slight greenish white pigmentation (Fig. 7). Growth, which was mucoid on smearing, was more luxuriant on agar No. 2, and also in broth.

The organisms were found to be still diphtheroid-like, but stained more deeply (Fig. 8). Club forms appeared in broth cultures. This stage grew equally well at temperatures of 30° C. and 37° C.

As the cultures aged the colony increased in size, with edges slightly undulate, and the organisms displayed a tendency to stain unevenly with Gram's. Pigmentation became definitely greenish, and the smears revealed the bacillary forms shortening to become coccoid, yet retaining the outline



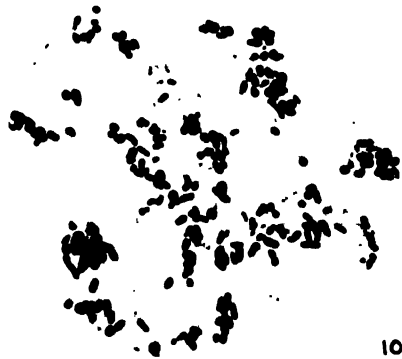
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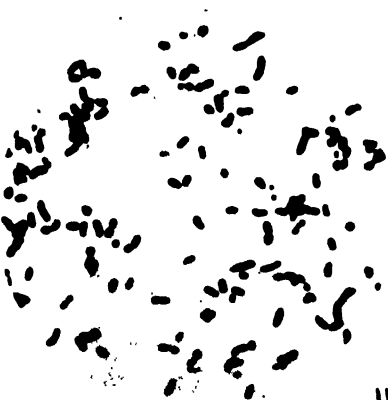
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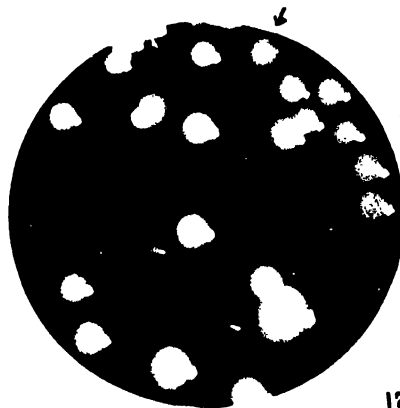
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11



12

FIG. 7. Typical "Stage 2" colonies. $\times 10$. FIGS. 8-11. Types of organisms found in "Stage 2" colonies; Gram's stain. $\times 1300$. FIG. 12. Typical "Stage 3" colonies. $\times 10$. (Notice two "Stage 4" colonies appearing.)

of their diphtheroid phase. The short rods and cocci stained deeply Gram positive, while the shadowy forms were Gram negative. Granules which took the Gram stain could be seen, some within the diphtheroid-like organisms, and some appearing to be free. (Figs. 9, 10, 11). After this stage, cultures were incubated at 37° C.

3. In the third stage, the colony was pea green in color, with little or no coloration on chocolate agar, and measured 0.5–1.5 mm. in diameter. (Fig. 12).

Coccoid forms predominated throughout the smears, a few short, thickened diphtheroids being found. (Fig. 13).

It has been said that from only one broth filtrate sown in K medium were streptococci recovered. These grew first as "stage 3" colonies after 35 days incubation at 30° C. and contained cocci in pairs and short chains. After transplanting four or five times they gave rise to colonies typical of strain No. 403. There was no diphtheroid phase found.

4. The pea green color of the colonies gradually became fainter and the diameter decreased until it measured about 1 mm. The centre was slightly raised and surrounded by a smooth ring. There was a slight green coloration on chocolate agar. (Fig. 14).

The organisms were cocci, usually larger than those of the original No. 403 strain, and occurred for the most part singly, paired and in very short chains. (Figs. 15, 16). A few remaining shadowy Gram negative forms could be seen. By means of four or five serial transplants to tubes of blood or chocolate agar and incubation at 37° C., a streptococcus was recovered which was found to be biologically similar in every way to the unfiltered strain No. 403. Occasionally it happened that at first the recovered streptococcus was inclined to produce slight acidity in salicin, but after one or two transfers it reverted to the same reaction as before filtration. Tested for virulence by the method used for strain No. 403, the recovered streptococcus proved to be non-pathogenic. This streptococcus has been re-filtered and recovered from filtrates of K medium, several times.

Above is described the series of "stages" which have been obtained in culturing the filtrates in most of the 20 experiments. In several of these, the colony development did not include the third and fourth stages, but the coccoid forms appeared in the whitish green type of growth. After seeding in nutrient, or Gray's broth (3), and re-transfer to chocolate agar or agar No. 2 at least ten times, a streptococcus identical biologically with strain No. 403 was recovered. It has sometimes been found that the diphtheroid, if left on agar No. 2 at 37° C. for six or seven days or longer, may change more quickly into the form of a coccus than it would when transplanted often to fresh medium. The transition from the short coccoid and diphtheroid organisms as illustrated in the photographs to cocci in chains may take place quite quickly. When transferred to blood agar No. 2 at 37° C. these forms have

been seen to develop into chains in four to five hours. As two of the photographs show, the streptococci could be seen appearing in the midst of the Gram positive and Gram negative diphtheroid and coccoid forms. (Figs. 17 and 18).

Study of the Diphtheroid

A belief that the diphtheroid, as found in the first visible growth on agar after filtration, was not the primary form of organism, led to the following study.

A series of cultures was made from a tube of K medium which had received 0.25 cc. of a filtrate of *Streptococcus* No. 403 grown in K medium, and filtered through a Chamberland L 3 candle. This culture tube had been incubated five days at 30° C. when a flocculence appeared. The structure of this flocculence was identical with that already described.

From this flocculence, transplants were made to a series of petri dishes containing agar No. 2, and to slides on which had been mounted thin blocks of the same agar. These were incubated at 30° C., and one of each examined every 40 min. for 42 hr. The smears from the cultures in petri dishes gave the most information. Examination was made under an 1800 magnification.

In three hours a few granules staining very deeply with a 1 : 10 dilution of carbol fuchsin, and intensely blue with Giemsa, were seen. With Loeffler's methylene blue, they resembled the metachromatic granules found in the *Corynebacteria*.

In four hours time, these granules had elongated. When stained, the original granular structure was still deeply colored, while the remainder took a lighter shade. Throughout the following 18 hr. the elongation continued, until the organism showed as a short, slightly curved, barred or granulated rod. (Fig. 4). There remained only a few of the deeper staining granules. In 48 hr. the colonies described as A type were visible to the naked eye.

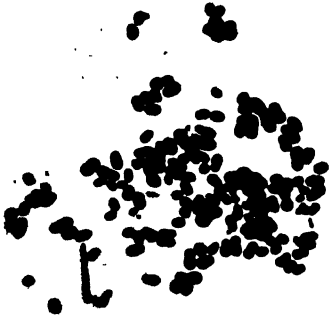
The diphtheroid organism differs from the *Streptococcus* No. 403 in its sugar reactions, which are as follows:

	Lactose	Mannite	Salicin
Acid	—	—	slight
Coagulation	—	—	—

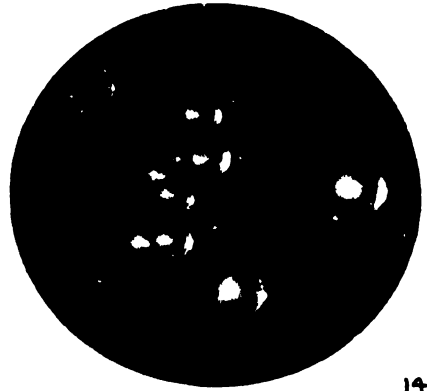
From the mucoid nature of the growth of the diphtheroid in its early stages, it would seem reasonable to expect the presence of a capsule. No capsule has been seen.

The diphtheroid organism was non-pathogenic when 1 cc. of a 24-hour-old culture on chocolate agar suspended in 10 cc. of sterile normal saline was inoculated into the peritoneal cavity of a mouse, as was also 1 cc. of the filtrate of a four days' growth of *Streptococcus* No. 403 in K medium when inoculated in a similar manner.

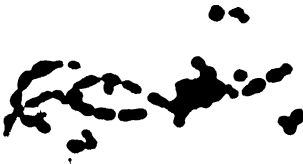
Inoculation of: (1) the stock, unfiltered *Streptococcus* No. 403; (2) the filtered and recovered *Streptococcus* No. 403; (3) the diphtheroid organism



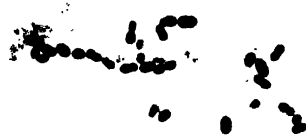
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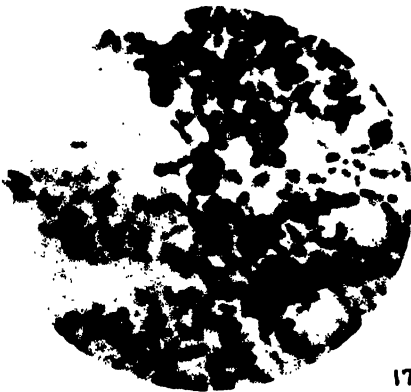
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15



16



17



18

FIG. 13. Type of organism found in "Stage 3" colonies; Gram's stain. $\times 1380$. FIG. 14. Typical "Stage 4" colonies. $\times 10$. FIGS. 15 and 16. Type of organism found in "Stage 4" colonies. (FIG. 15. Gram's stain. $\times 1360$. FIG. 16. Carbol fuchsin, 1 : 10. $\times 1380$.) FIGS. 17 and 18. Smear from "Stage 4" colonies growing eight hours on nutrient blood agar. (FIG. 17. Giemsa. $\times 1460$. FIG. 18. Gram's. $\times 1460$.)

recovered from filtrates; and (4) filtrate of *Streptococcus* No. 403 grown in K medium, was made into the udders of two cows. From clinical evidence it seems that in the great majority of cases, infection from one quarter of the udder is most unlikely to pass into the other quarter of the opposite side. That is, the right front quarter will not become infected from the left rear quarter, whereas the left front probably will in time. This is thought to be due mainly to the fact that the udder is divided into two halves by a double septum of fibrous tissue. Therefore the same cow was able to receive two different inoculations. In making these inoculations it was not expected that there would be any acute attack of mastitis, the disease being chronic in nature. Nor was it expected that there would be any changes involving the tissues, probably until after the next lactation period. But it was hoped that streptococci of the strain inoculated, not present already in the milk, would be recovered from it. However, there is not enough known about the mode of infection of the udder in chronic mastitis to justify the placing of much importance upon the results of the following experiments.

With all possible sterile precautions, milk was taken from the four quarters of the udder of two cows and plated out on chocolate agar. Forty-eight hours incubation at 37° C. produced only a few bacterial colonies, none of which were streptococci. Brom thymol blue tests were run on each quarter and proved negative.

1. In the first cow, inoculation was made into the teat canal of the opposite quarters, *i.e.*, the right front and left rear. Each received 10 cc. of an emulsion in sterile normal saline, of a 24-hr. growth on chocolate agar of *Streptococcus* No. 403.

The right front was inoculated with the stock unfiltered strain, and the left rear with the filtered and recovered strain.

Every 48 hr. milk was taken from all four quarters and plated out on chocolate agar. In five days, the two quarters which had been inoculated with the streptococcus showed a light green reaction with Brom thymol blue. In ten days time from the date of inoculation, streptococci identical with strain No. 403 were found growing on the plates planted from the milk of the right front and left rear quarters of the udder.

2. The second cow was inoculated by the same technique as was described above. An emulsion in 10 cc. of sterile normal saline was made from a 24-hour-old growth on chocolate agar of the diphtheroid-like organism recovered from filtrates of strain No. 403 in K medium, and inoculated into the left rear quarter of the udder. The right front quarter was inoculated with 10 cc. of the filtrate of a 72-hr. growth of *Streptococcus* No. 403 in K medium. The filter used was a Chamberland L 3.

In 11 days the culture from the milk of the left rear quarter showed streptococci, and in 14 days the cultured milk from the right front quarter also showed cocci in short chains. These proved biologically identical with strain No. 403.

Discussion

In all, 20 filtration experiments on the filter-passing properties of a strain of *Streptococcus* known as No. 403 have been completed. This organism was obtained from a case of chronic bovine mastitis. Out of this number 18 (90%) of the cultures in K medium have been filtered under the stated conditions, and under those conditions a streptococcus identical in every way with the unfiltered strain has been recovered.

From the same number of nutrient broth cultures filtered under the same conditions, only once has the original strain No. 403 been recovered. This recovery was obtained from a dilution of broth filtrate planted in K medium.

Steck (14) has discussed the possibility of there being a relationship between the diphtheroids and streptococci found to be present together or separately in the diseased or healthy udder of cows. He mentions Klimmer's suggestion that there might be a transition of diphtheroids into streptococci, and also refers to Diernhofer's finding of diphtheroid forms in cultures of streptococci. Steck has studied these diphtheroids and streptococci of the udder, and found them to be biologically different, though he considers that there may be some relationship between them during the course of the disease.

Culturally and morphologically, these diphtheroids are almost identical with the diphtheroid organisms which have been described in this paper.

Recently a report has been made by Evans (2) of forms of streptococci appearing in material and cultures from cases of epidemic encephalitis. She describes spore-bearing rod forms, some of which resemble the diphtheroid-like organisms found in cultures from the filtrates of *Streptococcus* No. 403 grown in K medium, though no bodies similar to those termed "gonidia" have been seen on the walls of the diphtheroids. The nearest approach to the formation of such gonidial structures might be found in the place wherein the diphtheroid forms partially lose their Gram positive properties, and appear granular, or as a Gram negative sheath surrounding a Gram positive coccus. This is illustrated in Fig. 9 where these Gram positive granules may be seen in the shortened forms.

Evans (2) concludes with this statement, "Life cycles are a law of nature; Algae, fungi and protozoa—the plant and animal groups standing next higher than the bacteria—exhibit marvellous life cycles. It is unreasonable to think that a law of nature which becomes more and more complex with the descent in the scale of life, would be suspended in its lowest known forms."

By means of motion microphotography, a recent study of *B. shigae* growing on various types of media has been contributed by Wyckoff (16). Using K medium and medium containing lithium chloride, the various "life cycle forms" were found to occur. Wyckoff does not consider that there is evidence in his work of stages in a life cycle, or that the "quick reversions" indicate the presence of filterable forms. He cannot yet offer an explanation of the "slower reversion".

TABLE III

SUMMARY OF FILTRATION EXPERIMENTS

Ex- peri- ment no.	Medium used	pH of fluid fil- tered	Filter candle	Time between filtration and first growth sign, days	Time between filtration and final recovery, days	Medium from which organism was recovered	Final result
1	K medium 1* Broth	6.2 7.6	New Berkefeld "N" New Berkefeld "N"	7		Organism died out	- -
2	K medium 1 Broth	6.7 7.6	New Berkefeld "N" New Berkefeld "N"	3	24	K filtrate in K medium	+ -
3	K medium 1 Broth	7.1 7.6	New Berkefeld "N" New Berkefeld "N"	3	25	K filtrate in K medium	+ -
4	K medium 1 Broth	6.0 7.6	New Chamberland L 3 New Chamberland L 3	4	32	K filtrate in K medium	+ -
5	K medium 1 Broth	6.3 7.2	Used Chamberland L 3 Used Chamberland L 3	4	31	K filtrate in K medium	+ -
6	K medium 2 Broth	6.8 7.4	New Chamberland L 5 New Chamberland L 5	6	30	K filtrate in K medium	+ -
7	K medium 2 Broth	7.0 7.5	New Chamberland L 5 New Chamberland L 5	7	35	K filtrate in K medium	+ -
8	K medium 2 Broth	7.2 7.4	Used Chamberland L 5 Used Chamberland L 5				- -
9	K medium 2 Broth	7.4 7.5	Used Chamberland L 5 Used Chamberland L 5	5	33	K filtrate in K medium	+ -
10	K medium 2 Broth	6.4 6.4	New Chamberland L 3 New Chamberland L 3	5	32	K filt.: in "K" and broth	+ -
11	K medium 2 Broth	6.6 6.6	New Chamberland L 3 New Chamberland L 3	4	34	K filtrate in K medium	+ -
12	K medium 2 Broth	6.8 6.8	Used Chamberland L 3 Used Chamberland L 3	5 35	33 39	K filtrate in K medium Broth filt.: in K medium	+ +
13	K medium 2 Broth	7.0 7.0	Used Chamberland L 3 Used Chamberland L 3	4	35	K filt.: in "K" and broth	+ -
14	K medium 2 Broth	7.2 7.2	Used Chamberland L 3 Used Chamberland L 3	3	32	K filtrate in K medium	+ -
15	K medium 2 Broth	7.4 7.4	New Chamberland L 3 New Chamberland L 3	5	35	K filtrate in K medium	+ -
16	K medium 2 Broth	6.4 6.4	New Chamberland L 5 New Chamberland L 5	6	34	K filtrate in K medium	+ -
17	K medium 2 Broth	6.6 6.6	Used Chamberland L 5 Used Chamberland L 5	5	36	K filtrate in K medium	+ -
18	K medium 2 Broth	6.8 6.8	Used Chamberland L 5 Used Chamberland L 5	7	31	K filtrate in K medium	+ -
19	K medium 2 Broth	7.0 7.0	Used Chamberland L 5 Used Chamberland L 5	6	35	K filtrate in K medium	+ -
20	K medium 2 Broth	7.2 7.2	Used Chamberland L 5 Used Chamberland L 5	3	24	K filtrate in K medium	+ -

*Indicates the number of the Tyrode solution used in the K medium.

The intention is now to quote each of the four postulates set up by Zinsser and see if they have been in any way fulfilled by the experiments performed with *Streptococcus* No. 403.

I. "It must be possible to filter a suspension of a well-defined pure culture of bacteria through a filter which holds back the characteristic forms; and these original forms should not appear in the filtrate or in cultures made with adequate amounts of the filtrate on suitable media after incubation of at least two or three weeks."

The requirements of the first half of this postulate would appear to have been met. Of course, as has been noted elsewhere, the suspension fluid, *i.e.*, K medium diluted with physiological saline, may have proved more favorable to the passage of organisms than nutrient broth diluted in a like manner. This being so, it would be fairly reasonable to expect that the control, *B. prodigiosus*, should pass through the filter pores at least occasionally. This was never found to be the case. The second half of the postulate seems also to have been covered. Filtrates were never found to contain characteristic streptococci. They contained no organisms at all, that could be identified. The amounts of filtrate planted seem adequate, and the media, blood and serum agar, also suitable; the original streptococcic forms were not recovered for at least two weeks, usually longer, and then after having passed through a diphtheroid phase.

II. "There must be evidence of growth of some kind in cultures made of this filtrate, but without evidence of the presence of the original normal forms."

The flocculence occurring in tubes inoculated with filtrate is taken as evidence of growth, inasmuch as organisms were recovered only from such tubes, and these organisms did not resemble streptococci in their morphology.

III. "The culture of the filterable or minute form must be carried in a series of several generations."

This postulate cannot be considered as fulfilled. The flocculence was carried through only two generations and then planted on blood agar. From this transfer streptococci were eventually recovered after passing through the aforementioned diphtheroid-like phase.

To the requirements of this postulate might be added the further test of a second filtration. That is, the filtrate culture containing flocculence should be re-filtered, and the second filtrate distributed as was the first. The attempt could then be made to recover the organism in its original form from the cultures of this second filtrate.

IV. "It must be possible to recover the normal forms from such successive 'filterable' attributes'."

In the light of the third postulate this fourth one has only been partially fulfilled. The normal forms of streptococci have been recovered from filtrates of growth in K medium, but not after successive passages of the filtrate

cultures from tube to tube. The suggestion may be offered that the slow recovery is probably after all only latent growth. This may be so; but in that case it would seem more reasonable to expect the normal cocci to appear and reproduce, than to find a diphtheroid phase giving place slowly to coccoid forms.

In conclusion, it may be said that no consideration whatever is given here to the suggestion of some workers that ultramicroscopic viruses may be identified with "filterable forms" of bacteria. Nor is it maintained that these filter-passing forms of a strain of streptococcus form a filterable stage of a bacterial life cycle, though the possibility is not excluded. Such evidence as there is seems to point to the presence of a viable particle in the filtrates of growth in K medium. The nature of this particle has not yet been explained satisfactorily. Whether it be a filterable phase, an altered or a degenerated condition brought about by some action of the medium upon the bacterial cell, we do not know. At the present time there do not seem to be sufficient facts offered and confirmed by investigators of the problem, to warrant any definite conclusion as to the existence or non-existence of a regular bacterial life cycle including a filterable phase.

Acknowledgments

The writer's thanks are due to Dr. E. A. Watson and Dr. C. A. Mitchell for their interest and advice; to Dr. L. J. Rhea and Dr. E. G. D. Murray of Montreal, for their criticism of the manuscript; and to Mr. H. L. Boyd of this Institute for his trouble in making the accompanying photographs.

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THE STRUCTURE AND BEHAVIOR OF UTRICULARIA PURPUREA¹

BY FRANCIS E. LLOYD²

Abstract

This paper presents an account of the more important structural features of a representative of a New World group of species within the generally accepted genus *Utricularia*, namely, *U. purpurea*. These species have been relegated to the genus *Vesiculina* by Barnhart, and the evidence here presented supports his views.

A general description is given of the habit and habitat of the plant, in which also the particular points of its form and structure are set forth. The important features of the internal anatomy are given.

The structure and behavior of the trap are described. It is shown that, while the mechanical working of the trap is in some respects strikingly different from that of the other species outside *Vesiculina*, the same fundamental plan of structure is adhered to. A detailed account of the structure of the door and of the threshold and their manner of action is given.

Utricularia purpurea is the sole representative in eastern North America of an exclusively* neo-tropical group of floating, or loosely anchored, submerged plants, in this having much the habit of *U. vulgaris*. Kamienski, in his analysis published in Engler and Prantl, includes the species under the section *Lentibularia*, associating with them *U. vulgaris* and some similar forms, and separating these from the *Megacista* (Sect. VIII) on the one hand and from the *Parcifolia* (Sect. X) on the other. This segregation and association, when viewed in the light of knowledge of the structure of the bladders (traps, as I prefer to call them), appear highly unnatural, however useful they may be in practical taxonomy. Barnhart (1) refers the *U. purpurea* segregate to *Vesiculina* Raf. on other grounds than the structure of the bladders—a confirmation of my conviction that, if any segregate of plants now inclosed in the genus *Utricularia* should be separated, certainly *U. purpurea* and its associates deserve this distinction. My own opinion is based chiefly on a study of the traps and, in addition to the immediate purpose of this paper, I shall try to show the evidence for my contention. For the rest, I propose to afford a more intimate account of the structure and behavior of the trap in *U. purpurea*, at the moment altogether lacking from our records.

The material used was collected in a small lake eight miles west of St. Jerome, Quebec, where my friend, Professor Marie-Victorin, had found it years previously. The name of the lake, Lac à la barbotte, serves but meagrely to identify it among the hundred others of the same name.

The plant is apparently not entirely freely floating, but is more or less anchored in the muddy bottom—possibly because of the sinking of shoots (especially winter buds) in the fall to settle in the mud. Viewed in the mass,

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* Barnhart (1) tentatively included F. Mueller's *U. tubulata* of Australia under *Vesiculina* Raf. Through the courtesy of Dr. A. B. Rendle, I have been able to examine material of Mueller's original (and only) collection, and can state quite definitely that his plant has not the characters of the *purpurea* type and it is highly probable that it conforms to the *vulgaris* type.

it is an unattractive plant, looking more dead than alive, brown and muddy in color. It is only on finding the ends of the shoots to be supplied with spreading verticils of branches each bearing a trap at the end, when young beautifully circinate and pale green in color, that one realizes that the plant is alive. The older parts of the shoots are purplish in color and devoid of traps, they having been shed by abscission. Add the fact that the plant is clothed with numerous glandular trichomes secreting mucilage to which stick all manner of minute forms and debris, and the rusty, ill-conditioned look is explained. It grows in shallow water, forming a zone of sorts just at the edge of the water-lily zone. It seems to flower in shallower water where it can mass more abundantly.

Previous studies of the traps of this or related species have been made by Goebel (7), Luetzelburg (15), Dean (4), and myself (12). Goebel described the curious trichomes found in a group issuing from a knobby projection near the middle point of the door in *U. purpurea*; Luetzelburg confirmed this for a South American species which he called *U. elephas*, because of its prominent trunk-like curved rostrum extending from the ventral side of the entrance. Thereto he added notes on the shape and structure of the door (of *U. purpurea*) indicating the forwardly flexed rim of the free door edge, but inadequately. He described also the threshold and front surface of the door of *U. elephas*. Dean, as a zoologist, was more concerned with the kind of food caught, but incidentally described quite briefly the trichomes of *U. purpurea*. He pronounced against the idea that the action of the trap is spasmodic, thinking that the door acts as a passive valve under which small animals crawl. The door is so weak as to allow such animals "to fall in of their own weight", he avers, all of which we shall see to be wrong. His interpretation of the functions of the valve hairs—namely, those of the door—were made to harmonize with his views of the passive character of the door. For my own part I have examined previously a species similar to, if not identical with, *U. elephas* Luetz., sent to me in preserved condition by Dr. Hoene, of Sao Paulo, Brazil. From it I was able to see clearly those structures together with the positional relations thereof, which enabled me to compare them directly with the corresponding ones in such species as *U. vulgaris*, *U. gibba*, etc. As my observations have been confirmed and amplified by study of living material of *U. purpurea*, I need not recount them here. I may say, however, that the details of structure and function are so different from those of *U. gibba*, etc., that a separate and full treatment is justified.

Form of the Plant

The plant consists of an axis of rather uniform thickness (about 1 mm. in diameter), tapering very slightly towards the apex. At intervals arise verticils of secondary axes (four to six in the verticil), these in turn verticillate, but becoming dorsiventral by the suppression of some of the tertiary axes. The axes of the younger verticils are all strongly circinate, and each bears at its end a young trap enwrapped within the roll. There are no "leaves" The

apparent radial symmetry of the plant is betrayed by the structure of the central cylinder which, as will be seen, is bilaterally symmetrical, as also by the evident bilateral symmetry of the winter bud. The purplish hue, less pronounced at the growing ends, is due to anthocyanin contained in the sap of the inner cortical cells and in the inner of the two courses of cells in the walls of the trap.

The winter buds (turions), by no means as highly specialized as those of *U. vulgaris*, are made up of verticils of compactly crowded circinate branches, separated at short distances by slightly developed internodes. The branches all bend upwards from the morphological lower side of the system.

The axes are dimorphic—one sort being widely spreading with shorter internodes, the other having longer internodes and much shorter branches.

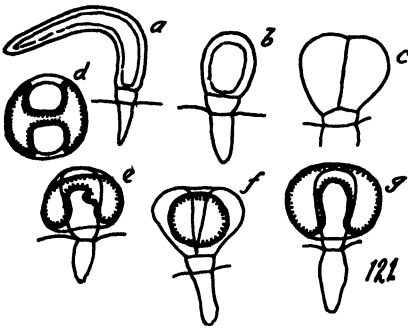


FIG. 1. Trichomes occurring on the surface of *U. purpurea*: a, sickle-shaped trichome, always bent downwards (backwards); b, oval mucilage secreting trichome; c, oil secreting trichome; d-g, various aspects of this trichome with the oil lying between the secreting capital cells and their common cuticle.

The epidermis is crowded with three-celled trichomes of three kinds, a unique situation among the Utriculariae. The most obvious is an elongated, sickle-shaped trichome which is very abundant, giving the surface a furry appearance (Fig. 1). Scattered among these is a second in much smaller numbers—a sessile trichome with an oval capital. Both have a thick-walled capital cell. The third kind is a low sessile trichome with a two-celled capital. The two cells eventually separate longitudinally but remain covered by their common cuticle, which forms a spherical investment and becomes filled with a colorless fatty oil.

Lodged between the cuticle and the two cells which secrete it, the oil takes a curious form, due of course to the shape of the cavity and to the fact that the walls are not wetted by the oil. I have extracted this oil with ether and proved it to be fatty. It has no odor, or at least not distinguishable from the general odor of water plants. The ether extract takes up the chlorophyll in solution. These three trichomes are morphologically a unit with the other forms found in and on the trap, consisting of a basal cell (epidermal) inserted between larger epidermal neighbors, a middle strongly cutinized small cell (mid-cell) and the frequently non-cutinized capital, of one or two cells according to sort. The whole surface of the plant is coated with mucilage produced by the trichomes; we may suppose by the two kinds, aside from the oil-secreting. All sorts of foreign matter adhere to the mucilage so that it presents a dirty appearance.

The trichomes are produced very quickly. Only the extreme tips of new axes are smooth. The earlier to develop are the oval mucilage and the oil trichomes, which appear to occupy nearly the whole surface; a little later the sickle-shaped trichomes make their appearance, on the outer flanks of the curves of circination first (Plate I-2).

Anatomy

The chief axis is cylindrical, gently tapering to the apex. The epidermis is composed of elongated cells, five times as long as broad. Between the ends, and usually at three-angled contacts, are inserted narrow fusiform cells, which are the basal cells of the three-celled trichomes, of which (as mentioned above) there are three kinds. There are no stomata. Under the epidermis there are three courses of parenchyma cells, constituting a cortex, which is broken up by large longitudinal air spaces (Plate I-1), each separated from its neighbor by radiating plates of a single layer of cells, penetrated intercellularly by lacunae permitting communication between the longitudinal air spaces, which are interrupted along the internodes by occasional oblique partitions. The air spaces thus formed are much larger than in *U. vulgaris*. The radial partitions are three to five cells deep. They abut on a starch sheath surrounding the vascular tract. In the starch sheath I can observe no Casparian spots, though they are evidently present in *U. vulgaris*, as Hovelacque recorded. The vascular tract has a diameter about one-fifth of the entire axis, and is composed chiefly of thin-walled, elongated elements with transverse walls, called "fibres primitive" by Hovelacque. In *U. vulgaris* these cells have much thickened walls. Whatever their mechanical virtues, they are the only mechanical elements present. The xylem may not be present at all—most frequently not. If present, it is in an eccentric position, marking the upper, ventral side of a very obscurely dorsiventral structure.* When present, it is a very poorly developed trachea with weak thickenings and very difficult to identify in transverse sections. Very occasionally, one finds a short length of trachea in a node, though the central cylinder may be devoid of xylem. The phloem occurs in isolated strands, a periclinal series of which lies against the endodermis, while, within, a few others lie scattered without obvious plan. The peripheral strands lie alternately with the cells of the endodermis, in the extreme angle between which lies a single longitudinal series of parenchyma cells, conspicuous for their dense contents. These I find in other species examined (*U. vulgaris*, *U. cornuta*) and have not before been noted. Hovelacque does not mention them. In common with the other species of *Utricularia* thus far studied, in the vascular system the xylem and phloem are dissociated and without any constant relation to each other beyond the indication of dorsiventrality indicated by the eccentric position of the xylem. The xylem is quantitatively almost negligible, though this does not mean that there is no movement of water. The established fact that the walls of the trap can transfer water from the inside to the outside, as observed by Brocher (2), suggests that this may be going on everywhere in the plant, but it is a matter subject to investigation.

Each node and the internode above are separated physiologically by a layer of suberized cells crossing the parenchyma. This appears to be an abscission layer, but is not such in fact. No true abscission occurs at this point. The plant is gradually overtaken by acropetal decay, without separation of the internodes.

* As in *U. neottiioides* (Goebel, 8).

Structure of the Trap

Each of the verticillate branches normally bears at its end a trap. Just below the trap the branch is suddenly narrowed into a slender stalk on which the trap swings quite freely. When the plant is moved about in the water, the traps are waved this way and that. Abscission takes place in this zone and thus the old branches are lacking traps. The continuity of the stalk of the trap and the branch is characteristic of the group and is to be seen in no other *Utricularia* (Plate I-3; II-19). Through the stalk the single vascular strand of the branch passes into the trap and extends along the dorsal edge as far as the upper lip of the entrance, where it ends abruptly. There is no vascular tissue along the ventral edge (Plate II-26).

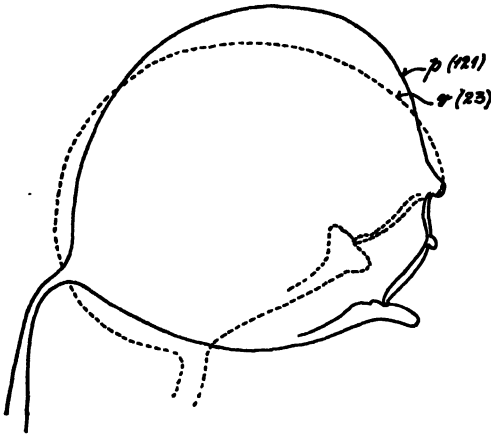


FIG. 2. Silhouette of *U. purpurea* (p) superimposed on that of *U. vulgaris* (v).

distinctive, best described as laterally compressed galeate. Its form is connected with the fact that the threshold lies parallel with the wall on which it stands, and is not rotated during growth as in other species. The dorsal marginal zone is arched over to meet the ventral zone to form the entrance. But the form can be understood only by means of a figure (Fig. 2; Plate I-3).

In color the trap is light green with a tinge of purple in the inner course of cells of the tissues especially in the region of the threshold. It is also exceptionally translucent, so that the interior structures may be more easily discerned than is usual in other groups. The surface is thickly clothed with the three kinds of trichomes mentioned above, the longest being curved backward, toward the stalk. Much mucilage is secreted and entangled by the trichomes, so that an abundance of detritus sticks to the surface.

When looking at the door from in front, one sees that the entrance is fairly oval, with a margin which lies in one plane, and this set somewhat

The trap itself is of peculiar and elegant form, which is quite distinct

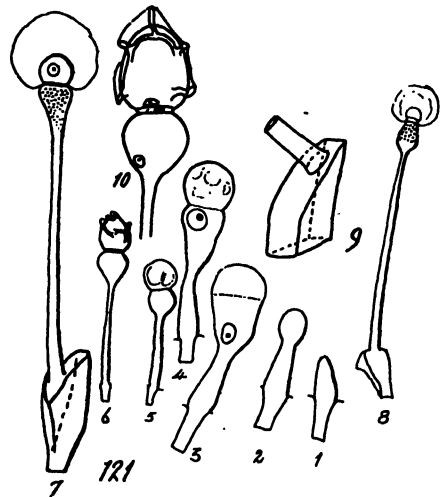


FIG. 3. Developmental stages of the tubercle trichomes, in the order of the numbering. Nos. 1-4 on twice the scale of 5-8; 7, 8, the two kinds of trichomes in the mature state; 9, basal portion of the trichome, enlarged. Note that, in No. 6, the cuticle is expanding being raised above the surface of the capital cells in ridges; shown also in 10.

obliquely with reference to a longitudinal axis, say one passing through the middle point of the entrance and the insertion of the stalk. Slight differences in position occur. The entrance is closed by the door and threshold, which lie somewhat within, leaving a free zone of approach clothed with short clavate trichomes, which project radially toward the middle point of the entrance (Plate I-5). Looking at the door itself, we observe at once a prominent tubercle occupying a position slightly above the middle point and bearing a radiating cluster of trichomes of two kinds (not one kind only, as has previously been supposed). The basal cell is long, slender and club-shaped. The walls near the top in the expanded portion are prominently pitted. This is surmounted by a very thin (Fig. 3), disk-shaped mid-cell, and this in turn supports a spherical gland cell with a ballooned cuticle. The gland cells contain a few chloroplasts. The two kinds of trichomes differ in the shape and proportions, as shown in the figures (Fig. 3; Plate I-6, 8). Those with the smaller end cells occupy a more peripheral position. The tubercle in which these trichomes arise is composed of the expanded bases of the basal (stalk) cells, and is part of the outer course of the cells of the door, as will be made plain beyond.

These radiating trichomes are analogous to the tripping hairs so characteristic of the *vulgaris* type of trap (Lloyd, 12), but differ not only in form but in their lack of rigidity. They may be bent most easily, oftentimes without springing the trap. Because of their size and position, however, a small animal may scarcely approach the door without impinging on these hairs, and often the merest touch is sufficient to spring the trap, with the consequent capture of the animal. I shall enlarge on this point in due course.

In order to examine critically the door, it must be dissected away. When lying in water it retains approximately its natural curvature, that of about a quarter-sphere. When flattened out it is nearly semicircular (Plate I-7), but departs from this form much more than does the door of *U. vulgaris*. This is related to the

width of the threshold, which is narrow in *U. purpurea*, and to the position of the threshold, of which more later. For the sake of more ready description, the accompanying diagram is given (Fig. 4). The index letters mean as follows: *b a a' b'* is the lower free edge of the door which rests on the

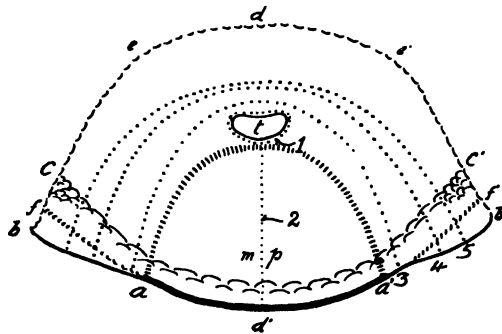


FIG. 4. Diagram of door flattened out: *a, a'*, middle reach of door edge heavily beaded; *ab, a' b'*, lateral reaches, not beaded; *b*, inner angle coincides with the inner angle of the threshold; *a f b*, the lateral area of door surface in contact with the threshold; *c c'*, position of the upper edge of the velum when the door is in position; *a (t) a'*, middle piece of door; *t*, tubercle; *b d b'*, edge of door attached to the walls of the trap; *l*, line of flexure around the tubercle; *2*, line of flexure along the axis of the middle piece; *3-5*, successive lines of flexure during opening; *e, e'*, points at which the door turns backward along the side walls of the trap.

threshold; $b d b'$ is the edge attached to the wall of the trap (the cells are therefore cut transversely along this line); $a a'$ is the middle reach of the door edge which is beaded and rests along the middle zone of the pavement epithelium of the threshold; $a b$ and $a' b'$ are the lateral reaches which traverse the pavement epithelium of the threshold to reach its inner angles; the zone marked off by $a b d t a' b'$ is the outer hinge zone; $a t a'$ is the middle piece of the door and the line 2 is a line of flexure along a longitudinal hinge; $e a b$ is a lateral area which turns back and is attached to the trap wall along $b e$; $f a b$ is a triangular area of the door which rests flat against the threshold, and not edgewise; $c c'$ indicates the position in which the velum lies against the door when in position of closure. 1 is a circular line of rotation of the tubercle, made possible because the tissue around and especially above the tubercle is thin (Plate II-16), obtaining a marked flexibility of the door in this region. The presence of this hinge-like region permits the rotation of the tubercle under impact on the trichomes sufficient to release the door edge from its emplacement, as we shall see.

Histology of the Door

We have adopted a terminology above to enable us to describe the histology of the door with relation to its movements.

It is composed of two courses of cells, continuations of the outer and inner epidermis of the trap wall, in this agreeing with the rest of the genus. It is also singularly translucent, if not quite as transparent as glass, so that, owing also to the thinness of the structure and the curvatures present, it is difficult to focus easily on either course of cells, especially the thinner ones. The walls of the other course shining through confuse the picture.

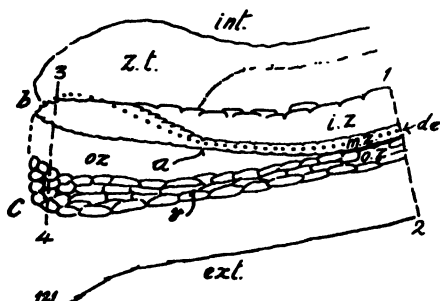


FIG. 5. Diagram of one-half of the threshold. $o z$, $m z$, $i z$, outer, middle and inner zones; $a b c$, as in Fig. 4; $z.t.$, zone of bifid trichomes. Section along 1-2, see Plate-fig. 22; along 3-4, see Plate-fig. 23. The position of the door edge when in position is shown by the heavily dotted line de to b ; v , velum; $int.$ and $ext.$, interior and exterior of the trap.

The best approach is to examine the door in sagittal section; the cut will then go through the tubercle (Figs. 7, 8; Plate I-6). This evidently separates two regions. The upper is, in section, in the form of a sigmoid flexure (Plate II-14). This is the outer hinge, but its action is more complicated than that of the outer hinge in the *vulgaris* type (Lloyd, 13). It will be seen that the two courses of cells alter their depth from the insertion above to the tubercle, the outer course of cells becoming thicker and the inner correspondingly thinner. The upper moiety of the hinge bends more readily outwardly, the lower inwardly; it is here that the maximum

bending occurs when the door opens (Figs. 8-A, 8-B). If a median strip of the door is cut free in water it springs forward (Plate I-8, 9), but when plasmolyzed

with 0.5 *N* potassium nitrate it returns only part way backward to its original position. This experiment demonstrates the region of maximum bending outwardly and inwardly. The tubercle is composed of enlarged cells of the outer course (Fig. 8; Plate II-16), while the juxtaposed inner course of cells is very thin. This, with the co-operation of a thin area (in the lower moiety of the door) around, but especially above, the tubercle, allows it a movement of rotation, very important in releasing the lower edge of the door from its firm emplacement. The thin region below the tubercle quickly passes over into the thicker portion to form a massive middle piece (Fig. 4), the cell courses of which are of equal thickness, except that the outer course becomes thinner toward the lateral regions where the door is attached to the wall (Plate I-10, 11) and where maximum bending occurs with the opening of the door. This extensive middle piece is identified with a much smaller analogous region in the door of the *vulgaris* type (Lloyd, 13). The thin region surrounding the tubercle may be equated with the central hinge of that type. The doors of the two types, *purpurea* and *vulgaris*, are thus seen to be conformable fundamentally, though widely different in details of structure.

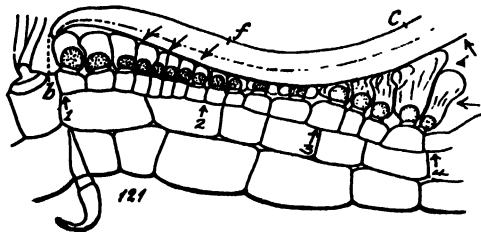


FIG. 6. Section along 3-4, Fig. 5, shown diagrammatically: 1-2, the tilted surface of the pad area of the middle zone; 2-3, the area between the velum and pad near the end of the threshold; 3-4, the velum; c, f, b, as in Fig. 4; the arrow points below f indicate the direction of pressure of the door surface when the trap is set; those at the right, the water pressure.

The free door edge is stiffened, but only throughout the width of the middle piece, by a forward-turning bead (Plate II-15). This fits into a groove (the middle zone) of the threshold when the trap is set. Its form, too, is important in relation to the velum, as will shortly be explained. The bead is made up of thickened cell walls of the door edge region (Plate I-6; II-15) (Luetzelburg, 15).

The large middle piece (Plate I-11) is seen to be uniformly thick except for a middle groove and composed of two courses of cells of equal depth. This part of the door, under the natural conditions of opening, does not bend sharply, but suffers first a shallow longitudinal folding, followed by a simple inversion of curvature from being bowed outwardly to inwardly. The maximum curvatures occur in the outer hinge above and below the tubercle, but more above, and around the corresponding lateral zones (Plate I-10). When the door is open the tubercle is therefore displaced inwardly (Fig. 7-B). The opening thus procured is circular, or nearly so (Fig. 7-C).

Having regarded the sagittal section, we are now enabled to study the aspect of the door as we face it from the outside, first *in situ*. Looking into the oval entrance (Plate I-5) we note at once the beautifully regular curvatures of the component cells. Above the tubercle (Plate I-7) they lie in paraboloidal curves, the more marked to the eye because of the intercellular spaces, more

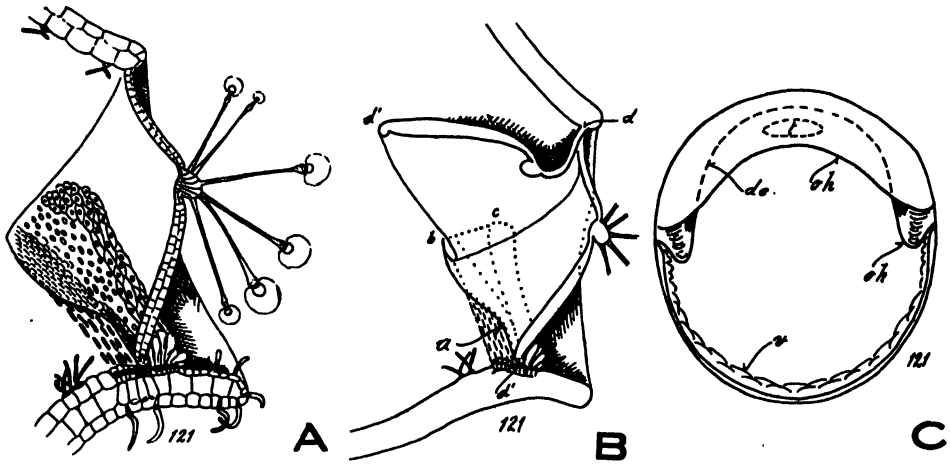


FIG. 7. A, Diagram of entrance split sagittally (cf. Plate-fig. 6), the door in position when the trap is set. The door treated as if transparent, so as to show the position of the threshold. B, same, showing the position of the door closed and open; lettering as in Fig. 4; C, front view of entrance when the door is open; oh, folded edge of outer hinge; de, door edge; v, velum.

or less gas filled, which signalize the rows of cells. The whole upper part of the door partakes of this character. Below the tubercle the pattern is continued by the curving rows of cells starting at the midline, where the cells are isodiametric, and passing downward and then upward, the cells now elongated in the direction of the curves of arrangement (Plate I-12, 13; II-25).

In harmony with the above described behavior, the character of the cells is the following. Regarding the outer course of cells first, we note that in

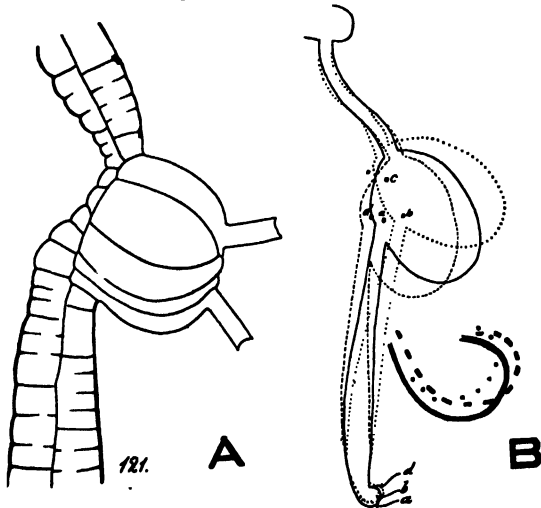


FIG. 8. A, sagittal section of door in the region of the tubercle. B, model to show the effect of movements of the tubercle on the position of the door edge; a, closed position; b, d, any other position on either side of the door in normal position.

these, in the regions of maximum bending, the lateral walls are markedly zigzag and are supported by many broad rods placed in the radial walls, chiefly at their angles (Plate I-13; Fig. 9). The same features occur in the outer course of cells of the door in the *vulgaris* type, but in *purpurea* are even more striking in their development (Lloyd, 13). This kind of cell is confined to the central hinge area, where it is nearly isodiametric. As the central hinge area merges into the outer hinge, the walls run in more strictly radial fashion, are less zigzag, and the rods

are less conspicuous. In the middle piece and extending laterally from this, the outer course cells are elongated in the direction of the curves of arrangement and agree in form almost exactly with the underlying cells of the inner course. Their radial walls are supported by numerous but small and inconspicuous rods, but display almost nothing of the zigzag form (Plate II-25).

The inner course cells are elongated in the direction of the curves of structure; only in the middle line of the middle piece are they isodiametric, conformably with the cells of the outer course. The walls are somewhat thicker than those of the outer course, and again are supported by numerous rods, which, however, are usually round and break up the walls into bays. The direction of these walls changes from bay to bay, running a very slightly indirect course. In the midline of the middle piece, the rods of the there isodiametric cells have large strongly developed rods correlated with a maximum bending capacity along this line (Plate II-25). The outer walls, forming the inner door surface, are corrugated; the corrugations running roughly parallel to the line of attachment of the door to the trap wall are most pronounced and conspicuous in the outer hinge area (Plate I-12). We have seen them in their greatest degree of development in the *vulgaris* type, in which the corrugations are very regular and deep, lending to the whole inner surface of the door the aspect of being marked off in concentric circles (Kruck, 10, Lloyd, 14). In *U. purpurea* however, they are so vaguely regular that no such appearance emerges—indeed can scarcely be observed at all, except near the line of attachment of the door (Plate I-12) where they display their maximum development, and are chiefly characteristic of the outer hinge, where, owing to the “bellows” structure (as Ekambaram has called it), they insure the maximum bending capacity of the outer walls. The particulars of structure for *U. vulgaris* have been fully described in the previous paper (Lloyd, 14). In that area above the tubercle where the door is bent in a sigmoid flexure, we find the maximum degree of regularity and development; where indeed the flexures of the door on its opening are pronounced.

Briefly stated, the plan of the door structure is one with that of the *vulgaris* type, but, correlated with the position of the tubercle, which bears the tripping mechanism, the proportions of the various areas are different. The minute structure of the component cells is the same also qualitatively, the degree of development of rods and infolds or corrugations being correlated with the particular flexures of the door, which, after presently considering the structure of the threshold, we shall be in a position to elucidate.

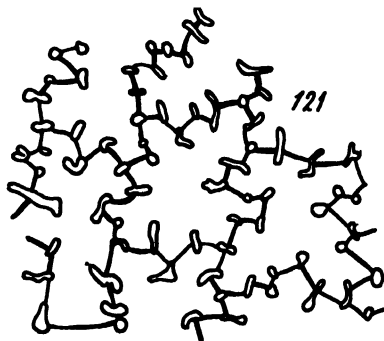


FIG. 9. Cells of the outer course of the outer hinge of the door above the tubercle (cf. Plate-fig. 13).

The Threshold, its Anatomy and Function

(Plate II—21–24)

The threshold in *U. purpurea* and its close relatives (*U. elephas*, *cucullata*, etc.) differs in the remarkable feature that it lies parallel to the wall from which it springs and is scarcely raised. For the purpose of emphasizing this comparison I have inserted Fig. 2, in which the bare outlines of the two types, *vulgaris* and *purpurea*, are superposed. It is this feature which underlies the difference in form of the two kinds of trap, one which is not immediately apparent to the casual observer. The flatter ventral outline and deeply arched dorsal profile are related thereto and are the result of different developmental behaviors.

Viewed from a position on the axis of the trap, the threshold is seen to lie on the inside surface of the entrance to the trap, a short distance within, equal approximately to its own width (0.2 mm.). It is nearly semicircular (Fig. 7–C). The surface of the threshold is a band of glandular epithelium, forming a pavement, widening somewhat towards its ends (Figs. 5, 6). The pavement is composed of the capital cells of closely packed three-celled trichomes, which for other species, especially *U. vulgaris*, have been described by several observers, from Hovelacque on. All previous observers have supposed them to be alike, whereas there are in *U. purpurea*, as in *U. vulgaris* and other species, three zones, the outer, middle and inner, in which the characters and functions of the component trichomes are different (Plate II—21–24, 28). The outer and middle zones are coterminous with the threshold; the inner zone is shorter and forms a lenticular patch lying between the inner zone and a zone of densely packed bifid trichomes (Plate II—21–23).

The outer zone is composed of capital cells with large swollen cuticles, and occupies a narrow band in the middle reach of the threshold spreading out fanwise toward the ends of the threshold. In this spreading region (*oz*, Fig. 5; between 2 and 3, Fig. 6) an inner triangle has capital cells whose expanded outer membranes are scarcely if at all cuticularized (they are not appreciably stained with Sudan III) but are sufficiently bloated to occupy the whole area (Fig. 6), though the capital cells themselves appear very loosely scattered. In front of this and running along the whole front edge of the threshold is the velum, the capital cells of which have much ballooned and highly cuticularized membranes whose function is to heap up against the door edge when the trap is in set condition. Thus they block the entrance of water which would be especially prone to occur along the lateral reaches of the door edge where the triangular surfaces *a b f*, *a' b' f'* (Fig. 4) lie against the threshold owing to the door edge traversing it from point *a* to point *b* to reach the inner angle of the threshold at *b*, where the door edge articulates with the walls of the trap.

The middle zone is narrow along its middle reach (*m z* as far as *a*, Fig. 5), bowed backwardly, and toward each end expands into an elongate oval patch forming a pad tilted outwardly (between 1 and 2, Fig. 6). The surface is of small, firmly tessellated capital cells. The narrow middle reach receives the

pressure of the door edge; the lateral pads offer resistance to the door surface included approximately in the triangles afb , $a'f'b'$, Fig. 4. The tilting of the surfaces of the pads toward the outside permits the door to be cramped inwardly so that the door surface lies firmly against the threshold and comes into contact with the broad velum found in this region. The condition is illustrated in Fig. 6.

The inner zone is made up of capital cells with enlarged cuticles strongly cuticularized presenting a surface which is tilted outwardly, that is, in the same sense as the pads of the middle zone. Anatomically indeed these pads may be considered as a continuation of the inner zone, but I have preferred to designate these areas in terms of function. The inner zone has the function of resisting the inswing of the middle reach of the door edge. When the door opens the edge must glide over the inner zone.

The threshold therefore is seen to be a band of tissue the component cells of which are structurally adapted to hold the door firmly in position when the trap is set, and to make and keep it water-tight so that on exhaustion of water there is no inleakage.

The Action of the Door

Plate I—5-9; II—14-17.

Fig. 7 shows a sagittal section of the door, and Fig. 8, in greater detail, through the tubercle. This is composed of the hypertrophied bases of the trichomes, and is a much swollen area of the outer course of cells. Fig. 8-B is a diagram of a putative mechanical equivalent. Such a model can be only imperfect and no account can be taken of the delicate elasticity of the original. The point to be emphasized is that a slight rotation of the tubercle in any direction can have the effect of raising the lower edge of the door ever so slightly. This movement need not occur in precisely the middle of the door edge, as the model would indicate. Perhaps we should say that all that is required is a momentary distortion of the door edge. Such distortion will produce at the moment of occurrence an asymmetry to resistance of water pressure, this being all that is necessary. For, by virtue of the pressure of water on the door (owing to the reduced internal pressure—usually called negative pressure), the slightest asymmetry in position of the door edge allows the release of pressure against the ridge formed by the swollen cuticles of the inner threshold zone. The pavement epithelium, the door itself, and indeed every bit of surface of the trap is coated with mucilage which lubricates the parts so effectively that the contingent surfaces slide readily on each other. Once the shift of the door edge is accomplished, the water pressure swings the door in fully, or at least as fully as it is equal to (Fig. 7-B). This depends of course on the time which has been allowed for recovery from a springing. According to my own observations, it requires about two hours for the exhaustion of the water from the interior of the trap to the extent illustrated in Plate II—19 and 26—longer or shorter, according to individual traps. This is in contrast with *U. vulgaris*, which can exhaust itself sufficiently

for renewed action in a half-hour or less (Merl, Czaja). In *U. purpurea* the exhaustion proceeds till the sides of the trap become appressed together (Plate II-26). This is brought out most clearly in a trap in which on being tripped in air the bubble inclosed becomes distorted during exhaustion (Plate II-19).

It will now be appropriate to consider the nature and amount of disturbance of the tubercle trichomes which can cause sufficient distortion of the door edge to effect its release and so upset the "unstable equilibrium" (I quote Brocher), leading to the opening of the door. The following conclusions rest upon a very large number of experimental observations on fully developed but variously sized traps. The largest are about 3 mm. long; the small ones a half of this. They show, moreover, a very great difference of delicacy and this becomes more marked if in a culture there has been an accumulation of mucilage and adherent debris.

The ideal trap, if such an expression may be permitted, is one which responds most readily. This response is purely mechanical, as I have attempted to show in a previous paper (14). This species, *U. purpurea*, lends itself most strikingly to the elucidation of this question, for with care the trichomes of almost any trap may be disturbed by contact with a needle point without procuring a response. It is usual to be able to brush aside the trichomes repeatedly, provided direct impact is avoided. It is thus easy (much more so than in *U. vulgaris*) to prove that there is no reception or transmission of stimulus by the trichomes (as held by Kruck for *U. vulgaris*). As to the nature of the contact which is effective, one may say as follows. In a minority of cases the merest touch of a flat-sided needle point on the top of one trichome (so far as one could see with a binocular) at once released the mechanism. The delicacy is so great that one is usually surprised by the promptness of the response. This is the ideal condition. In other cases the trichomes may be displaced by a gentle upward, downward or lateral stroke of the needle, either without procuring response or with more or less prompt action. Frequently the action follows on the sudden resumption of the normal position by the trichomes after the needle has passed over them, or, briefly, on the back-swing. Or the needle point may be inserted between the trichomes and be pressed on the tubercle itself, with response. In others, response follows only on more vigorous impact. In common with *U. vulgaris*, lifting the plant out of the water results in the release of the mechanism in many traps by the water films accompanied by the ingulfing of air, as Brocher and others observed in *vulgaris*. The mere movement of the plant in the water often effects release, so that, because of the many traps present, often the small traps—young ones, yet unopened—are swallowed by large ones. In fact, a good way of experimentation is to use minute traps instead of a needle point. The more sensitive traps when put in action by the contact of a small trap (say 0.6 mm. long) promptly swallow it (Plate I-4). Now as the traps swing delicately on their pedicels, one gets an impression of extreme mechanical sensitivity. Obviously the impact of minute water animals (crustacea, worms, mites) suffices to actuate the traps.

The inswing of the door under pressure of the outside water is possible only because that organ is elastic, bending easily in every direction. Aside from the inherent physical properties of the cellulose of which its cell walls are composed, the resistance of the door to such water pressure arises from the fact that the door edge is a little longer than the threshold, that is, the line $b\ d'\ b'$ (Fig. 4) is longer than the line $f\ d'\ f'$ along the threshold. The result is that the door cannot swing in without buckling. This takes place along the middle line (Fig. 4-2) of the middle piece of the door, reaching from the door edge to the tubercle (Plate II-25). As soon as this release is accomplished, the middle piece as a whole becomes bowed in the sense opposite to that of the normal position, so that the flexure beginning in the midline is transferred to the outer hinge. This it follows just above the tubercle, this structure being bent inwards with the middle piece; it thus disappears from view in the profile of the open trap (Fig. 7-B). The path of flexure above the tubercle lies in the area where the inner course of cells is thinner than the outer course. At the consummation of the inward movement, the opening must be a nearly circular one (Fig. 7-C), since the entering column of water will act as a solid rod, except for the fold of the outer hinge above the tubercle. This seems always to cut off a chord of the arc. By measurement, however, a trap about 2 mm. long can receive a glass bead about 0.57 mm. in diameter, this slipping in with no impedance. The trichomes might seem to be such, but the turning inward of the tubercle inclines them all inwardly and they are easily brushed aside by an entering object, aided by their general mucilaginous character.

The rate at which the door action takes place is such that the eye cannot follow any details of the movement. By means of motion pictures taken at normal rates, we know that the entire movement takes place within one-sixteenth of a second. Within this brief period the door swings in fully (Fig. 7-B) and then out again to its original position (Fig. 7-A). It is the swiftest movement in the plant kingdom, excepting perhaps ciliary movement. Naturally, one cannot make statements about the flexures of the door except as one may study the movement under pressure, preferably of a spherical object, unless slow motion photography is used. I have succeeded in this only in another species as yet, namely *U. vulgaris*, on which I shall report elsewhere.

Development of the Trap

We turn finally to the development of the trap, with special regard to the features peculiar to the subgenus as evaluated here. The course of development for the trap of *Utricularia* in general, as typified by *U. vulgaris* and nearby species, was worked out by Meierhofer (16). In brief, an ascidium develops at the end of a lateral organ. The dorsal lip of the ascidium develops into the door and the ventral into the threshold and lower lip of the entrance. The point of articulation of the two lips is the point b (Fig. 4); $b\ d'\ b'$ of the door edge at first coincides with the inner margin of the threshold but continued growth lengthens it so that it comes to fit the curved line, running obliquely along the threshold, as already described (b to de , Fig. 5).

In the species under consideration, the differentiation of the structures of the trap which are peculiar to it begins very early. From the beginning, the outer surface is thickly clothed with the three kinds of trichomes above described (Fig. 10). A fully developed large trap is 2.5 to 3 mm. in length;

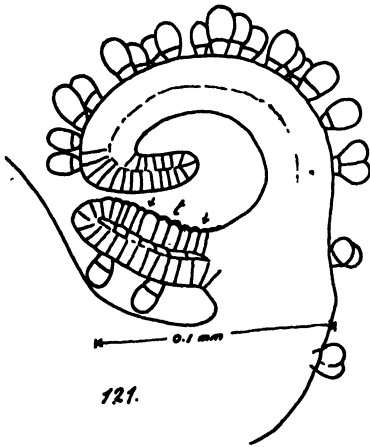


FIG. 10. Sagittal section of trap 0.1 mm. long.

but by the time it has reached a length of 0.3 mm. all the structures are distinct (Fig. 11), though not in a definitive condition. Of particular interest is the differentiation of the door, including the trichomes and tubercle, and of the threshold. In a trap 0.1 mm. long the amount of differentiation is scarcely enough to distinguish it from other species; nevertheless, the form of the ventral lip betokens the absence of that rotation which distinguishes, e.g., *U. vulgaris* (Meierhofer, 16, Fig. 40). The door still lacks the stigmata of the species. The trichomes of the outer surface are numerous; the oil-bearing few in number, the sickle still absent (Fig. 10).

A trap 0.3 mm. long. At this point in development the door projects inwardly and the lower free edge is shorter than the threshold. The trap is still tightly closed. The tubercle is scarcely visible, except that its cells have at this moment become short, cone-shaped projections. The curvatures of the door are distinct, but the cells undifferentiated, or but little so. The threshold is laid down, but no secondary differentiations marking the zones have appeared. The row of bifid absorbing trichomes backing the threshold (Plate II-22) is represented by a slightly raised cushion formed by the growth of the band of inner epidermis, just within but slightly separated from the threshold. From this point on, the changes are as follows.

The trichomes of the door tubercle (Fig. 3; Plate I-5-9; II-18, 20, 27). From a short, conical condition (Figs. 1, 3), in which the basal part is only very slightly enlarged, the protuberant portion elongates into a tubular tapering elongation furnished with a round knob, which later elongates and becomes pyriform, the slender end tapering into the stalk. A transverse wall now makes its appearance, after which both cells swell into nearly spherical moieties. A second transverse wall now is formed just above the first, cutting off the mid-cell—a longitudinally very thin cell, to which the cuticle remains adherent. The end or capital cell now enlarges to its maximum, forming a thick cuticle, and in shape ovoid ($18 \mu \times 25 \mu$) (Plate II-20). The cellulose wall finally becomes globose leaving the large cuticle free. At first the cuticle wrinkles (Plate II-27), but subsequently expands and becomes filled with mucilage, forming (in *U. purpurea*) a large spherical bladder or balloon. This is the large trichome. The smaller stops short at the stage in which the swollen end is cylindrical, with two transverse walls (Plate II-27). The

capital cell has a less swollen cuticle. The outer end of the basal cells differs in shape also from that of the large trichome, as shown in Fig. 3. This region of the walls thickens and becomes pitted, affording an irregularly punctate picture (Plate II-18). At the latter stage of development the basal cell elongates fully, and the capital cell with its surrounding loose cuticle swells to its maximum. The tubercle is thus composed of the conjoined basal fractions of the basal cells of the door trichomes, which are part of the outer course of cells,

The enlargement of the cuticles of the capital cells is worthy of remark. We already know that the cuticles of the outer zone of the threshold which furnish the velum arise by secondary expansion, forming relatively enormous balloons. To a less extent the same occurs in the cells of the inner zone, remaining (in *U. purpurea*) intact and forming an impedance to the inswing of the door. It appears to occur also in the oil glands of *U. purpurea* (Fig. 1). That the membranes

are cuticular has been demonstrated by the usual staining methods; they stain deeply with Sudan III and IV. In the velar membranes there is evident growth from the base, as shown by the presence of double membranes (Plate II-28) at the sides of the balloons. At the other extreme of behavior there is the entire abjection of the cuticle, as occurs in the bifid and quadrifid hairs and the external glands, and in many other special cases. The genus is, in fact, notable for the various behaviors in regard to the cuticle of the glandular trichomes.

The threshold. At the early stage studied (0.3 mm. long), the threshold is differentiated as a band of glandular cells with bacilliform capital cells, their longer axes running transversely with respect to the trap, longitudinally with respect to the threshold. Those of the outer zone become deeper and, at the time the tubercle trichomes form the first transverse wall, begin to throw up the velar membranes, which soon reach their full expansion (Plate II-28) before the door is ready to come into its definitive position with its free edge along the middle zone. This zone is a lineal depression occupying the middle of the threshold, and is lined by two or three rows of compact capital cells. The inner zone develops the swollen cuticles somewhat later.

During all this time the trap remains completely closed but by the time the tubercle trichomes approach their definitive condition (somewhat later than as seen in Plate I-27) the mouth of the trap begins to open and the door to swing outwardly into its final position. Because of the position of the door during the growth of the trichomes, these all point inwardly and, as seen

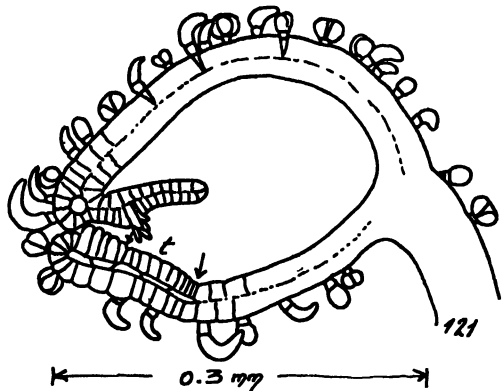


FIG. 11. Sagittal section of trap 0.3 mm. long. The tubercle trichomes just appearing.

during the opening of the trap, they maintain the flexures for some time, straightening out only when the final stage of adjustment is reached, the trichomes then assuming the radiating positions of the mature condition.

Taxonomic Position of the Group of Species Exemplified in *U. Purpurea*

The characters of structure presented by *U. vulgaris* and *U. purpurea* stand in such sharp contrast that one can hardly suppress surprise that the segregation which has been in general use remains accepted to-day in spite of Barnhart's studies (1). In Kamienski's treatment, he puts into the section *Lentibularia*, Gesn., *U. flexuosa*, *U. neglecta*, *U. vulgaris*, *U. minor* and *U. intermedia*. In the section *Megacista* DC., *U. stellaris* and *U. inflata*, and in the section *Parcifolia*, *U. exoleta*, *U. gibba* and *U. obtusa*. In addition to these, there are two species, one of which, *U. neottioides*, Kamienski places in a fourth section, *Avesicaria*. The other *U. rigida* Benjamin, of tropical Africa, is described by Stapf as being "nearly allied to" *U. neottioides* of Brazil, and, according to the description, has no bladders (traps). The name of the section indicates an opinion now known to be false, since *U. neottioides* has traps (Luetzelburg, 15, Lloyd, 12) and it is probable that *U. rigida* has also, though at present there is no evidence on this point.

The studies of Barnhart have led him to regard the segregates recognized by him as distinct genera. While an insufficient knowledge of the group does not permit me to make any far-reaching criticisms, it seems perfectly clear to me that the segregate *Vesiculina* has fully as much, I think rather more, to justify it than the generally recognized genus, *Polypompholyx*. The whole plant body, including the traps, is so very distinctive, and some of the differences I have indicated above. Grouped with *U. purpurea* on these grounds are the South American species, such as *cucullata*, *elephas*, Luetz., *U. myriocista*. The traps of these are all of the same character structurally, only *elephas* having a superficial distinction in a proboscis-like projection from the lower dorsal edge of the entrance (Luetzelburg, 15, Lloyd, 12). The traps may be briefly characterized, for taxonomic purposes, as having a door with a nearly central knob bearing radiating trichomes with widely inflated capital cells. This separates them at once from all other Utriculariae. The other species included with *purpurea* by Kamienski fall into two groups. In one, the insertion of the door is at the outer margin of the entrance, as in *U. vulgaris*, *U. diploglossa*, *U. oligosperma*, *U. intermedia*, *U. stellaris*, *U. flexuosa* (cf. Goebel, 7, figure, 6); *U. mixta*, *U. geminiscapa* and others belong here. In the other, the upper edge of the door is set back by the development of an overhanging or projecting extension of the trap wall, so that the door is approached as through a vestibule, as in *U. gibba*. Of this type are *U. pallens*, *U. pumila*, *U. exoleta*, *U. neottioides*, *U. herzogii*, etc. It requires but little skill to make the distinctions as regards the trap structure if the traps are present, as, however, they usually are in these floating plants. The structure of the traps is, however, important in the classification of the other

species, many of which are represented in herbaria by material which is so scanty and frequently altogether lacking in these organs that we may well take to heart the remark of Stapf (18): "In fact, a really satisfactory classification of this genus will only be possible when living or carefully collected and preserved spirit material is at hand". This applies to all parts, which are so delicate that, once subjected to drying pressure, only a very inadequate idea of the original plant can be obtained. As all the species mentioned in the preceding paragraphs have been so collected, I am enabled to entertain some confidence in saying that Barnhart's recommendation to separate the *purpurea* species is vindicated.

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EXPLANATION OF PLATES

PLATE I

FIG. 1. Transverse section of an axis.

FIG. 2. Whole preparation of end bud of growing tip, showing early development of oil and mucilage trichomes.

FIG. 3. Silhouette of living trap showing the position of the door when the trap is set.

FIG. 4. A trap which has swallowed a young trap.

FIG. 5. Front view of the entrance of a trap, the door in position.

FIG. 6. Sagittal view of entrance. In this the door is not in its true position since the entrance has spread (cf. Text-fig. 6).

EXPLANATION OF PLATES—Concluded

PLATES—Concluded

FIG. 7. Somewhat over a half of a door laid out flat, showing the disposition of the cells (cf. Text-fig. 4).

FIG. 8. A median narrow piece of the door of a living trap, showing the maximum flexure when lying in water.

FIG. 9. The same on treatment with 0.5 N potassium nitrate.

FIGS. 10 AND 11. Transverse sections through the middle piece (Fig. 11) and the outer hinge at the same level (Fig. 10). Note the relative thickness of the cell courses, and the slightly thinner middle line of the middle piece.

FIG. 12. Inner surface of the outer hinge near its attachment to the wall. Note the lines indicating the infolds of the outer walls of the cells.

FIG. 13. Portion of the outer hinge obliquely above the tubercle, showing the outer course cells (with zigzag walls and heavy rods) overlying the radially elongated cells of the inner course.

PLATE II

FIG. 14. Sagittal section of the outer hinge and tubercle of the door, showing the change in dimensions of the outer and inner cell courses.

FIG. 15. The door edge, sagittal section, showing the beading.

FIG. 16. Sagittal section of the tubercle, showing the dimensions of the cells.

FIG. 17. The tubercle as it appears from in front.

FIG. 18. Top of a mature tubercle trichome, showing pitting in the expanded portion of the basal cell.

FIG. 19. The same trap, living, just after tripping, with an inclosed bubble of air (left) and two hours later, in the set condition. The collapsed sides (as in Fig. 26) have forced the air bubble to occupy the peripheral space.

FIG. 20. Young tubercle trichome with expanding cuticle, the capital cell not yet in its definitive form.

FIGS. 21–24. The threshold.

FIG. 21. Median region, looking down on the surface of the threshold. The various regions can be recognized in Fig. 22.

FIG. 22. Sagittal section of threshold. The regions: velum, middle zone (narrow and dished), the outer zone and the trichomatous zone can be equated with the corresponding zones in Fig. 21.

FIG. 23. Threshold, section through 3–4, Text-fig. 5. Reversed with respect to Fig. 22.

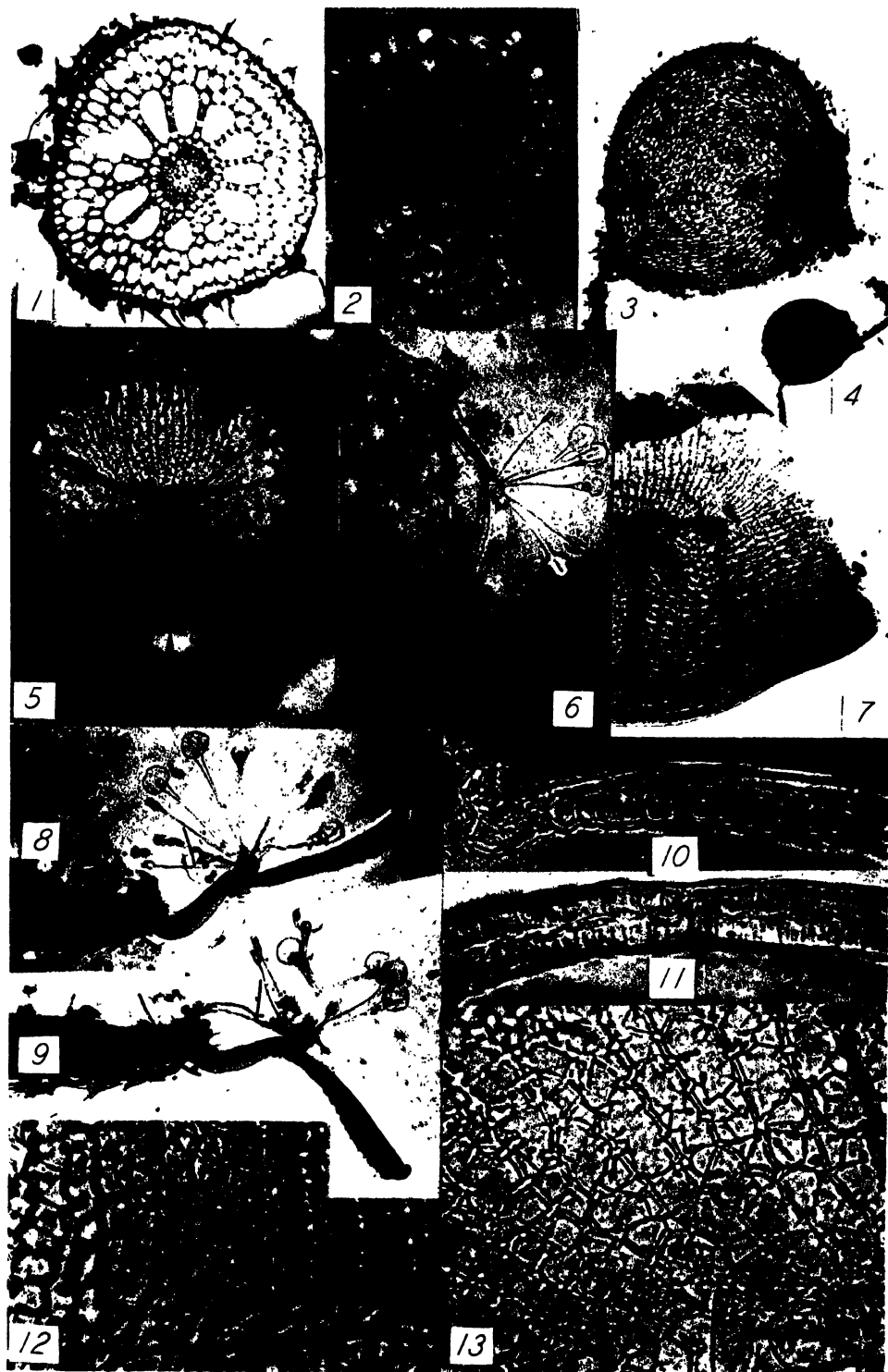
FIG. 24. View looking into the entrance over the lower portion of the threshold, showing the diaphanous velum.

FIG. 25. Surface view of a portion of the middle piece of the door to show the middle line of small cells, with the laterally spreading curves of cells of both courses superimposed.

FIG. 26. A set trap and the same immediately after tripping, living. Note that on account of the exhaustion of water, the sides of the trap (left) are mutually quite compressed.

FIG. 27. A young, still unopened trap showing particularly the door at this stage of development. The two kinds of trichomes are here well seen.

FIG. 28. The same stage as that in Fig. 27, but showing better the newly developed velum. The cuticles of the inner zone of the threshold are not yet fully expanded.





FUNGI ISOLATED FROM MANITOBA SOIL BY THE PLATE METHOD¹

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Abstract

The fungus-flora of 75 samples of surface soils from various parts of Manitoba has been analyzed. The population of fungi in these soils is abundant and varied despite climatic conditions which might appear unfavorable. Forty-four genera and 100 species of fungi have been identified, and the relative frequency of each determined. It has been found that the methods of soil microbiology give data regarding the parasitic as well as the saprophytic fungi in soils; that *Trichoderma lignorum* parasitizes certain other fungi, and thus may play a part in keeping in check the pathogenic fungi within a soil; and that the addition of chemical fertilizer to a soil results in a prompt and consistent increase in the fungus-flora.

Introduction

The activities of soil micro-organisms are unquestionably of importance to agriculture. Many bacteria and fungi are indispensable in the soil because they decompose organic matter to simple compounds that can be utilized by higher plants. Some are injurious because they bring about unfavorable chemical changes, or because they attack the roots of crop plants.

Bacteria, Actinomycetes, and Fungi* exist in the soil in enormous numbers; they are isolated commonly from Manitoba soils at the rate of 10,000,000 or more per gram of soil. Although no more than 1 to 3% of this total represents fungi, these are of such importance in the soil as to deserve especial attention.

Much work is necessary to establish the identity and relative frequency and importance of the soil fungi in various parts of the world. Manitoba is well situated for an investigation of this problem since the soils are of many and varied types; little work has been done upon soil fungi within several hundred miles; and the wide temperature and moisture changes in the soil make the results of interest. This publication presents a summary of the work undertaken to determine the identity, prevalence, and significance of fungi found in surface soils in Manitoba.

The isolation technique outlined by Waksman (25) and Brierley, Jewson and Brierley (3) has been followed. Each analysis was made on a composite of ten samples taken with a garden spatula to a depth of about four inches after the loose surface layer had been removed. The composite was mixed thoroughly in the field on a sterile canvas, transferred to a sterile paper bag and transported to the laboratory. A moisture test was run at once, and the

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*Fungi as used throughout this paper refers to the Eumycetes or true fungi belonging to the classes Phycmycetes, Ascomycetes, Basidiomycetes, and Fungi Imperfecti.

result expressed as percentage of the moisture-free soil. The sample, weighing about five pounds, was spread between sterile papers and air dried at about 28° C. for three days, after which it was pulverized, sifted and mixed thoroughly under aseptic conditions. Air-dry moisture was determined and the count recorded on a moisture-free basis. Twenty-five grams of the moisture-free soil was shaken in 250 ml. of sterile water in a litre jar on a mechanical rocker shaker for 30 min. One ml. of a 1 : 5000 dilution was placed in each of eight plates. Four of these were poured with Waksman's peptone-glucose acid agar (8) and four with Czapek's sodium nitrate-sucrose agar (8), both acidified just prior to pouring by the addition of 1 ml. and $\frac{1}{2}$ ml. respectively of 12.5% lactic acid per 100 ml. of medium. Incubation was at 25° C. for from three to five days depending upon the presence or absence of spreaders. Each count represents the average of the results on the eight plates, except in a few cases where spreading types made counting impossible on all plates, when the results on the remaining plates were averaged.

After counting, one plate showing good distribution of colonies, or in some cases one plate from each medium, was selected and each colony picked and transferred to a Czapek's (non-acidified) agar slant or in the case of *Mucor* types, which do not develop well on this medium, to Waksman's agar for study. The other plates were kept and any different-appearing or tardily developing colonies isolated at a later date and marked "extra". In addition three plates of a 1 : 100,000 dilution in sodium albuminate agar (8) and three of the 1 : 50,000 dilution in nitrate-sucrose agar (8), for counts of bacteria and Actinomycetes respectively, were examined for fungi after about 14 days' incubation and isolations made of colonies not recognized as common species.

Isolations were made from 75 samples covering the following soils: garden, orchard and sod, alfalfa and cereal fields at the Agricultural College at Winnipeg; a millet field and a summerfallow at Niverville, south of Winnipeg; a forest, a slough and sod and barley fields at Loni, about 60 miles north of Winnipeg; field and forest at Keewatin, just over the eastern boundary of Manitoba; and forest in the Riding mountains in western Manitoba. The most extensive study was that on two wheat plots carried on over a period of seven months starting April 1932. In the list of species presented below reference to the source is made when a fungus predominated in a particular type of soil. Since the significant differences in the fungus-flora of the soils studied are uncertain the isolations from the various soils are not compared in detail.

The surface soils studied had an average count of 125,000 fungi per gram, or 25 colonies on each plate of 1 : 5000 dilution. Thus about 15,000 colonies were examined macroscopically on the 600 plates for fungi, and an additional thousand or more colonies of fungi were examined on the 450 plates for bacteria and Actinomycetes. The list of species below accounts for the 2,565 transfers which were studied in test tube cultures.

Gilman and Abbott's summary (9) has proved exceedingly useful in this study, and also Waksman's works (23, 24, 25) and other publications, some of which are mentioned in the list of references.

Notes on the Groups of Fungi, and Acknowledgments of Assistance

Many soil fungi are difficult to identify. However, careful and continued study, utilizing when necessary various culture media, gives data by means of which most species may be identified from published descriptions. With some fungi it is necessary to have help from mycologists who have given intensive study to certain groups. The writers are glad to acknowledge with thanks the kind assistance of those mentioned below, who have so generously examined many cultures.

Myxobacterales, Acrasieae, and Myxomycetes are present in soils, but the special technique necessary to demonstrate the presence of these Myxothallophytes has not yet been applied in Manitoba.

The Phycomycetes are fairly common in soil, in this study representing 137 cultures or 5% of the isolations. All the Phycomycetes recognized belong to the Mucorales. Species of *Pythium*, *Saprolegnia*, *Olpidium* and other lower Phycomycetes are known to occur in soil, and some of them attack the roots of living plants. None of these fungi was obtained in this investigation, because the culture media used were not favorable for their development, and because of the greater relative abundance of spores and mycelium of other fungi in the soils examined.

The Mucorales often are troublesome on isolation plates because their rapid growth in an incubator at 25° C. overwhelms young colonies of other fungi. Fortunately, however, most Mucorales produce a thin growth on Czapek's agar, so that the slower growing fungi may be picked with the overgrowth to tubes of Czapek's agar, where they can often be identified, although plating for purification is necessary in some cases.

Drs. A. F. Blakeslee and Sophie Satin have reported upon several species of *Absidia*, *Syncephalastrum*, and *Zygorhynchus*. *Rhizopus* accounted for more than half of the Phycomycetes isolated.

Ascomycetes which produce asci in culture are rarely found in soil isolations. *Chaetomium*, *Sporormia*, and *Fimelaria* occasionally appear; they are probably more common in soils fertilized with barnyard manure. Yeasts and other lower Ascomycetes appear infrequently in acidified plates.

Basidiomycetes appear to be even scarcer than ascus-bearing fungi in soil isolations. One culture of a smut, a few cultures that might be mycelial stages of Hymenomycetes, and the *Rhizoctonia* stage of *Corticium vagum* were obtained. It might be expected that *Sporobolomyces* would occur in surface soils. Since the commoner species of *Sporobolomyces* are pink, six isolations of pink yeasts were tested, but none discharged spores from sterigmata as *Sporobolomyces* does.

Larger Basidiomycetes often grow conspicuously upon soil, particularly in forests. These fungi are, however, seldom recognizable upon isolation plates from soil.

Fungi Imperfecti constituted more than 90% of the isolations. Species of *Penicillium* are by far the commonest fungi in Manitoba soils, as in soils elsewhere. More than half (1404) of the cultures studied were *Penicillia*. Dr. Thom very kindly named the numerous cultures sent him. The writers may have made mistakes in identifying cultures to be the same as "authentic" cultures determined by Thom, but care has been exercised, and practically every culture has been examined microscopically as well as macroscopically, in comparison with named species and with the assistance of Thom's monograph (20).

Aspergilli were isolated infrequently. Dr. Thom has named a representative culture of each species herein reported.

Fusaria appear commonly in soil isolations. Dr. Sherbakoff identified several species of *Fusarium* and *Cylindrocarpon*, and Dr. W. L. Gordon of the Dominion Rust Research Laboratory at Winnipeg undertook the onerous task of comparing the various cultures, and of identifying them in consultation with Dr. Sherbakoff. Dr. J. E. Machacek, also of the Rust Research Laboratory, has examined critically a number of cultures of various fungi.

Mr. E. W. Mason of the Imperial Mycological Institute, Kew, has given much help with the Dematiaceae and Mr. S. P. Wiltshire of the same Institute has examined certain *Alternarias*. Professor H. H. Whetzel of Cornell University has reported upon several difficult fungi.

Professor J. H. Ellis of the University of Manitoba has given many valuable suggestions relating to soils.

The list of species which follows gives the name of the fungus, with a query where there is doubt as to its identity. The numbers following the name refer to the number of times the species occurred. The approximate percentage of all isolations is indicated whenever the fungus was found to the extent of 1% or more. In the cases of some of the less common species, the fungus was isolated partly or entirely from "extra" plates other than the one or two picked for the standard routine determinations; in such cases the number of "extra" isolations is given. Thus *Helminthosporium sativum* appeared three times in routine isolations, and four times on plates not picked, so the record is "7, four of which were extra". The notes given with species refer to certain diagnostic or other features that seem worthy of record.

List of Species

The fungi are arranged alphabetically under the groups indicated. All specific names are decapitalized.

I PHYCOMYCETES

Absidia glauca Hagem (2, i.e., identified twice only.) Both isolations are of the (+) race and produce zygosporangia with Dr. Blakeslee's minus test race. *A. glauca* is recorded by Gilman and Abbott (9) from soil in Idaho only in North America, and from three European countries.

Absidia orchidis (Vuill.) Hagem (4). The cultures fit the description of *A. orchidis* very well, but the name has not been verified.

Absidia spinosa Lindner (6, four of which were from sod.) A homothallic fungus with short-cylindric spores.

Absidia spp. (3, not definitely determined.)

Mortierella isabellina Oud. var. ***ramifica*** Dixon-Stewart (2, from forest soils.) Cultures produce a thin gray to drab growth, with branched sporangiophores bearing sporangia $15-20\ \mu$ in diameter, with small globose spores $2-2\frac{1}{2}\ \mu$. The fungus fits the description given by Miss Dixon-Stewart (6) for a fungus isolated from bush soils in Australia.

Mucor ?abundans Povah (4) A *Mucor* near *M. hiemalis*, but with sporangiophores more branched, spores $4-6 \times 3-4\ \mu$.

Mucor dispersus Hagem (1) Sporangia $20-70\ \mu$, some with only 3-4 spores; sporangium wall marked with short spines as in *Rhizopus elegans*; spores variable in size, from $7\ \mu$ to $15 \times 12\ \mu$, globose to slightly oval.

Mucor hiemalis Wehmer (11, especially in forest soil.) Sporangiophores mostly unbranched, but occasionally with one or two branches, sporangia $30-90\ \mu$, spores variable, mostly oval, often $8 \times 4\ \mu$, frequently smaller.

Mucor racemosus Fres. (9, the commonest species of *Mucor* in wheat fields.) Sporangiophores branched racemosely; spores fairly large, up to about $10 \times 8\ \mu$. The sporangia are encrusted.

Mucor ?sylvaticus Hagem (1) Spores of two sizes, $4 \times 3\ \mu$ or up to $9 \times 6\ \mu$, sporangia small, mostly about $30\ \mu$, chlamydospores present, often terminal.

Mucor spp. (3 unidentified.)

Rhizopus elegans Eidam (9, but see next entry.) Less vigorous in growth than *R. nigricans*, but with rhizoids well developed. Easily recognized by the "elegant wimperig stachelig" markings on the sporangia. Spores $5-8\ \mu$, roundish. Perhaps in reality more common in Manitoba soils than is *R. nigricans*. A number of cultures were paired in an effort to obtain zygosporangia, but none were produced.

Rhizopus nigricans Ehr. (67 cultures, over 2%, credited to this species, but at first it was not distinguished from *R. elegans*.) *Rhizopus* is common in soil and troublesome on isolation plates.

Syncephalastrum racemosum Cohn in Schroet. (12, in two isolations from wheat fields.) Cultures white, then yellow and finally dark brown. Sporangiophores branched, bearing globose heads of sporangia with spores globose, $3-5\ \mu$ or oval and up to $8 \times 4\ \mu$. Apparently not previously reported from soil.

Zygorhynchus heterogamus Vuill. (1, garden.) Zygosporangia comparatively large, $50-75\ \mu$, becoming black, with coarse rough markings. Spores from sporangia roundish, up to $4\ \mu$ in diameter.

Zygorhynchus ?moelleri Auct. Amer., non Vuill. (1, forest soil.) Zygo-spores $30-55\ \mu$, golden brown with pyramidal markings $4-5\ \mu$ high. Spores from sporangia $4-5 \times 2-3\ \mu$. Dr. Blakeslee states that this fungus resembles the form which has gone under the name *Z. moelleri* in America, which differs from the species called by this name in Europe.

Zygorhynchus sp. (1, forest soil.) Dr. Blakeslee writes that it is neither *Z. vuilleminii* nor *Z. moelleri*. The transfer kept at Manitoba has been lost. Waksman (23) reports *Z. vuilleminii* to be common in subsoils. Species of *Zygorhynchus* apparently are rare in surface soils in Manitoba.

II ASCOMYCETES

Chaetomium ?funicola Cke. (6, three of which were extra.) These cultures are placed here with some doubt. The perithecia produce setae which are not incrustated, sometimes branched. Asci not clearly seen, but the spores are often in aggregations and seem to have been produced in asci. Spores very abundant, sometimes $5-7 \times 3-3\frac{1}{2}\ \mu$, but often as small as $4 \times 2\ \mu$. The cultures produce dark floccose mycelia studded with perithecia.

Chaetomium ?spirale Zopf (1) Perithecia bear masses of spiral hairs, somewhat incrustated. Ascospores $11-15 \times 6-8\ \mu$, olive green, ellipsoid with narrowed ends.

Fimetaria fimicola (Rob.) Griff. and Seaver (1) Perithecium pyriform, nearly smooth; ascospores $18-22 \times 12-13\ \mu$, greenish, with a hyaline envelope.

Gymnoascus ?reessii Baranetz. (1, wheat field.) Culture purplish-red, producing numerous roundish perithecia $125-200\ \mu$ consisting of loose wefts of hyphae with ends projecting, and containing roundish asci $7-10\ \mu$ in diameter with 8 small oval spores $3-4 \times 2\ \mu$. This fungus is apparently a *Gymnoascus*, but does not fit the species *G. reessii* well.

Saccharomyces (?) spp. (9 cultures from routine isolations on acidified agar.) Some forms are pink, but in no case was *Sporobolomyces* found.

Sporormia fasciculata Jensen (2) Perithecia are produced readily on Czapek's agar. The asci are $40-55 \times 20-27\ \mu$, and contain eight four-celled spores which are $28-32 \times 6-7\ \mu$, brown. These spores readily separate into single cells $7-10 \times 6-7\ \mu$. The colony has a purplish and white appearance, and the perithecia may be aggregated into small clusters. Since the fungus resembles the description of *Sporormiella nigropurpurea* Ell. and Ev., described on dung, cultures were made on sterilized horse dung, upon which the fungus grows well, but the asci are shorter and wider than reported for *Sporormiella*.

Sporormia sp. (2) Cultures resemble the preceding species, having a purplish color. One isolation produced perithecia readily (possibly because the culture was contaminated), the other isolation developed perithecia only when yeast extract was added to the culture medium. Both produce asci longer than in *S. fasciculata*, being $70-110 \times 14-20\ \mu$, the spores $20-25 \times 6-7\frac{1}{2}\ \mu$, brown. The perithecia are single, not in clusters.

III BASIDIOMYCETES

Rhizoctonia solani Kühn (1, wheat field.) This fungus is known to be common in soils, but is not obtained often by the isolation methods used in soil microbiology. Pratt (17) isolated it five times from Idaho soils.

Ustilago sp. (1) Produces a rather slimy cream-colored growth, with areas of thin white mycelium. The spores or cells are mostly $8-24 \times 2-3\frac{1}{2} \mu$, and show budding. Dr. W. F. Hanna examined the culture and found it to resemble *U. zeae*, but it could not be determined definitely.

IV FUNGI IMPERFECTI

A. *Moniliaceae*

Acrostalagmus cinnabarinus Corda (5, three of which were extra isolations.) Colonies cinnabar or brick red, becoming sometimes white mycelioid. The conidiophore verticils are often 4-5 rayed, but sometimes have only two or three branches. The spores are mostly $3-5 \times 1\frac{1}{2}-2 \mu$, but a few spores can be found $6-10 \times 2-3 \mu$. This fungus seems to fit the species rather than the variety *nanus*.

Aspergillus flavipes (Bain. and Sart.) Thom and Church (25, 1%). This has proved to be the commonest *Aspergillus*, but was obtained almost exclusively from wheat fields. It produces a yellow growth with amber drops and a "forest" of long conidial heads.

Aspergillus flavus Link group (4, one of which was extra.) The cultures were found to produce kojic acid.

Aspergillus fumigatus Fres. (9, especially from old sod.) This *Aspergillus* occurs on old plant parts, and sometimes causes the death of young chickens. Cultures grow rapidly at 38° C.

Aspergillus niger van Tiegh. (3, one of which was extra.) This fungus is more common in soil in southern parts of North America. Werkenthin (27) found it to be the most frequent soil fungus in Texas; Le Clerg (14) reports *A. niger* to constitute more than 1% of the fungus-flora in Colorado soil; Miss Todd (22) has recently found it to represent about 40% of the surface soil flora in Oklahoma. *Aspergillus niger* is conspicuous by its rarity in surface soils in Manitoba. It is not easily mistaken or overlooked. It provides an example of a soil fungus with apparent variation in regional distribution.

Aspergillus okazakii Okazaki (2, wheat field.) A member of the *A. candidus* group.

Aspergillus ustus (Bainier) Thom and Church (6, one of which was extra.) The cultures are dark gray; considered by Dr. Thom to be perhaps between *A. ustus* and *A. insuetus*.

Aspergillus versicolor (Vuill.) Tirab. (1, wheat field.) Reverse of colony red to purplish.

Botrytis cinerea Pers. (3, one of which was an extra isolation.) The three cultures show the usual variation in spore and sclerotium formation.

Botrytis terrestris Jensen (3, one of which was extra.) Professor Whetzel agrees that this fungus is similar to, or identical with, the *B. terrestris* of Jensen (13), although he does not consider it to be a good *Botrytis*. The fungus produces a gray-brown restricted growth, with a more or less velvety surface and a few dark drops, cultures reddish-chestnut-brown below. Spores $3-4 \times 2\frac{1}{2}-3 \mu$, forming masses at the ends of the conidiophores.

Cephalosporium acremonium Cda. (19, nearly 1%.) The *Cephalosporium* which produces a pink to salmon, slimy growth on potato dextrose or malt agars, with narrow cylindric spores $3-6 \times 1-2 \mu$, is interpreted to be *C. acremonium*.

Cephalosporium ?curtipes Sacc. (13) A white fungus with spores in heads, $4-9 \times 2-4 \mu$. Spores rather small for *C. curtipes* as described.

Cephalosporium humicola Oud. (4) Apparently this species; conidiophores rather long, spores roundish or oval, $3-4 \times 2-3 \mu$. The colonies are rather paler in color than in *C. acremonium*.

***Cephalosporium* sp.** (11) Several times a *Cephalosporium* has been isolated which produces spores $6-12 \times 2-3 \mu$, grows well on Czapek's agar producing a wrinkled damp colony sometimes salmon, sometimes yellow, sometimes showing both colors. This fungus may be the same as reported by Waksman (23, p. 133) as "G.23".

Cylindrocarpon ?candidum (Link) Wollenw. (1, extra.) Culture white, producing cream-colored masses of conidia, $42-58 \times 5-6\frac{1}{2} \mu$, mostly three-septate.

Cylindrocarpon candidum* var. *majus Wollenw., probably (6, from wheat field.) Cultures somewhat darker in the variety. Spores $50-64 \times 5-6 \mu$, mostly three-septate, produced in cream-colored masses.

Cylindrocarpon didymum (Hart.) Wollenw. (11, especially in wheat field.) Cultures brown to yellow, sometimes pinkish, and usually with an odor resembling that of a broken young stem of maize. Spores produced in buttery yellow masses, one-septate, $20-28 \times 3\frac{1}{2}-4\frac{1}{2} \mu$.

Cylindrocarpon ?heteronemum (B. and Br.) Wollenw. (2, wheat field.) Cultures white to brownish, spores $16-20 \times 3-4 \mu$, commonly with one septum.

Cylindrocarpon ?macrosporum (Fres.) Wollenw. (4, wheat field.) Cultures brownish, chestnut brown on reverse; spores $20-40 \times 5-7 \mu$.

Dactylium dendroides (Bull.) ex Fr. (1, forest soil.) Produces a *Mucor*-like growth with verticillate conidiophores bearing three-septate spores $20-30 \times 9-12 \mu$. It is curious to note that the fungus on Czapek's agar produces the purple-red color characteristic of *Hypomyces rosellus*; on potato dextrose agar the culture is only very faintly pink. Not a true soil organism, but found in Manitoba upon old *Hymenomycetes*.

Fusarium coeruleum (Lib.) Sacc. (1, wheat field.) This fungus is a common cause of dry rot of potatoes in Manitoba.

Fusarium culmorum (W. G. Smith) Sacc. (16, all from cereal fields, and 9 of these from a plot which had been inoculated with the organism.) This fungus, parasitic to cereals, can be isolated by the methods of soil microbiology. An isolation was found to be parasitic to wheat, as recorded below.

Fusarium culmorum* var. *cereale (Cke.) Wollenw. (3 isolations from soil previously inoculated with cultures of *F. culmorum*.)

Fusarium equiseti (Cda.) Sacc. (2)

Fusarium herbarum (Cda.) Fr. var. ***avenaceum*** (Fr.) Wollenw. (1, garden.)

Fusarium moniliforme Sheldon (3 identified.) This *Fusarium* and its varieties are probably fairly common in soils. Found also by Henry (10) in soil from Brandon, Man.

Fusarium oxysporum Schlecht. (10, from garden and old sod, rarely from wheat fields.) Found frequently in Manitoba as a parasite in wilted potatoes. *Fusaria* belonging to the *elegans* section are common in soil, but several have not been identified.

Fusarium poae (Peck) Wollenw. (1)

Fusarium reticulatum Mont. (2)

Fusarium sambucinum Fckl. (2, wheat field.)

Fusarium scirpi Lamb. and Faut. (9, various soils.)

Fusarium scirpi* var. *acuminatum (Ell. and Ev.) Wollenw. (21, 1%)
Common in various soils. The cultures are floccose and bright red.

Fusarium solani (Mart.) var. ***martii*** (App. and Wollenw.) Wollenw. (9, found especially in wheat fields.) The cultures develop the typical green to blue color.

Fusarium sporotrichioides Sherb. (2, from soils with cereals.)

Fusarium vasinfectum Atkinson (24, 1%, especially from sod and alfalfa field, but also in garden, orchard and grain field.)

Fusarium vasinfectum* var. *lutulatum (Sherb.) Wollenw. (21, 1%, especially in garden.) Aerial mycelium is often replaced by a yellowish-pink mass of pseudopionnotes.

Fusarium vasinfectum* var. *zonatum (Sherb.) Wollenw. (1, wheat field.)

Geotrichum candidum Link (2) Cultures on agar are white or pale cream color, with a broadly spreading, rather velvety but thin mycelial growth, and with the ends of the hyphae breaking up into segments (spores) $5-12 \times 3-4 \mu$, cylindrical, ends obtuse. These cultures were carried as "*Monilia* sp." until tested on sterilized leaves, where small white tufts were produced, and the fungus seems to agree with *Geotrichum candidum* as recorded by Lindau (15) and by Sumstine (19).

Gliocladium atrum Gilman and Abbott (2) This fungus produces round *Gliocladium* heads with spores about $3 \times 2 \mu$ on penicillate conidiophores, but, as Gilman and Abbott state, the mycelium is so dark as to place it in the *Dematiaceae*.

Gliocladium catenulum Gilman and Abbott (1, an extra from sod.) Colonies white then green, with long heads of spores, reverse yellowish. Spores $5-8 \times 2\frac{1}{2}-3 \mu$.

Gliocladium penicilloides Corda (8) An attempt was made to separate in this species the paler, more cream-colored cultures with spores somewhat smaller, but unquestionably this "species" merges into *G. roseum*.

Gliocladium roseum (Link) Bainier (42, nearly 2%) Common in various soils. The cultures are white then salmon pink. Spores in heads, $4-7 \times 2\frac{1}{2}-4 \mu$.

Hymenula affinis (Fautrey and Lambotte) Wollenw. (13, from garden and wheat field.) This fungus produces a pale salmon, slimy layer, with abundant spores with one or two cells.

Metarrhizium sp. (28, 1%; all cultures except one from wheat fields.) This fungus is easily recognized by the production on Czapek's agar of a floccose mass of pure white mycelium, which later produces very dark areas of spores. The reverse is pale yellow. Spores $5-8 \times 2\frac{1}{2}-3 \mu$. Some species of *Metarrhizium* are parasitic upon insects. A specific name for this fungus has not been found.

Monilia ?geophila Oud. (1) This *Monilia*, with a bright yellow growth and conidiophores breaking up into spores, resembles *M. geophila* except that the spores are larger, being $6-10 \times 4-6 \mu$ on Czapek's agar. On gelatin the spores are variable, and the gelatin is liquefied.

Monilia sitophila (Mont.) Sacc. (2) This fungus grows so rapidly and conspicuously that it cannot be overlooked in cultures. Evidently it is not abundant in soil in Manitoba.

Penicillium albidum Sopp (1, from sod.) Determined by Thom as possibly this species. The culture is white at first, then green to olive green, zonate and radiately wrinkled, reverse orange to buff. Conidiophores enlarge considerably at apices.

Penicillium aurantio-brunneum Dierckx (3, millet field.) The cultures produce a brilliant amber color in the medium.

Penicillium braziliense Thom (9, one of which was extra.) This *Penicillium* is easily recognized by its chalky-white appearance, with usually a faint tinge of pink. The reverse of the culture is yellowish; one culture which remained for some months in the refrigerator developed a dark green color in spots on the reverse, and this color persisted in transfers.

Penicillium canescens Sopp (3, wheat field and forest soil.) This fungus, identified as "close to *P. canescens*", has been found previously in soil in Norway and England.

Penicillium carmino-violaceum Dierckx (27, 1%.) A beautiful species which colors the medium shades of red to violet.

Penicillium chrysogenum Thom (152, 6%.) *Penicillia* belonging to the *chrysogenum* series are common in soil. Drops are produced on the surface which are commonly yellow, sometimes nearly colorless. The reverse is yellow to brown. Milk is digested with production of a bright yellow color.

Penicillium ?duclauxi Delacr. (1, wheat field.) The fungus scarcely grows upon Czapek's agar, but produces a coremiform growth on potato dextrose agar.

Penicillium funiculosum Thom (11) Produces reddish coremia with masses of green spores; medium colored deep red.

Penicillium frequentans Westl. (11) Not found to be common in Manitoba soils. The cultures resemble those of *P. rugulosum* superficially, but lack the bright yellow margin in reverse.

Penicillium griseo-roseum Dierckx. Certain cultures agree with the description of this species, but are counted with the related *P. chrysogenum*.

Penicillium intricatum Thom (299, 12%.) This is one of the commonest organisms in the various soils examined. It produces a funiculose mass of hyphae at first white or gray to brownish and without spores, becoming smoky green from spores; reverse brownish.

Penicillium janthinellum Biourge (217, 9%.) Also very common in Manitoba soils. Thom (20) calls the *P. janthinellum* series "the 'soil' *Penicillia*".

Penicillium kapuscinskii Zaleski (1) Our culture resembles *P. intricatum*, and possibly we may have referred a few cultures erroneously to the latter species.

Penicillium lilacinum Thom (21, nearly 1%.) This species sometimes is striking, with a lilac surface and yellow reverse, but various shades of colors are produced by different isolations. Three cultures show the *Isaria*-like hyphal columns mentioned by Thom (20, p. 335) as occurring with the form *Spicaria violacea*.

Penicillium luteum Zukal, group (3, garden.)

Penicillium purpurogenum Stoll (4) The surface is yellow-granular at first, and the reverse is purplish to red.

Penicillium purpurescens Sopp (3, wheat field.) Dr. Thom writes that this fungus agrees with Sopp's description. Surface green, powdery in older cultures; reverse purplish to very dark; spores echinulate, $3\frac{1}{2}$ – $4\frac{1}{2}$ μ .

Penicillium restrictum Gilman and Abbott (45, 2%.) This fungus, with restricted growth and dark surface, is common. Usually the reverse is colorless, but in ten of our cultures the reverse is brownish.

A form that seems nearest *P. restrictum* is commoner: we have isolated it 95 times (nearly 4%), especially from forest soils. The spores are globose and rough; the colony is dark colored, often somewhat green. It differs markedly in appearance from the typical form in the thin growth that is rather powdery from spores and sometimes zonate.

Penicillium rugulosum Thom (55, 2%) Spores oval, slightly rough; colonies brilliant yellow at sides of slants in test tubes, and in reverse. Thom (20) reports *P. rugulosum* as a common parasite of *Aspergilli*. Tests were made by growing *P. rugulosum* with colonies of *Fusarium culmorum*, *Helminthosporium sativum* and *Rhizopus*, but in no case was parasitism by this strain of *P. rugulosum* evident.

Penicillium rugulosum* var. *atricolum (Bain.) Thom. Two or three cultures produce the restricted growth and paler reverse of this variety.

Penicillium simplicissimum (Oud.) Thom (26, 1%, especially from forest soil.) This species resembles one strain of *P. terrestre* superficially, but the conidiophores are different.

Penicillium spinulosum Thom (2) This monoverticillate *Penicillium* has rough spores and a dull green, loose conidial area.

Penicillium terrestre Jensen (307, 12%) This has been found to be the commonest *Penicillium* in soils cropped with wheat. One strain has a floccose surface, another is powdery; young cultures often have an aromatic odor resembling that of bananas. All cultures become gray when older. The reverse, on Czapek's agar, varies from colorless to buff, pink, or brownish.

Penicillium thomii Maire (66, nearly 3%, especially common in forest soil.) This variable fungus is characterized by the production of dense masses of sclerotia which may be salmon, pink, yellowish or brownish. The reverse is nearly colorless to brown. In some strains abundant pale greenish conidial areas are produced sooner or later, in others no noticeable conidial production occurs. The conidia are oval, on monoverticillate conidiophores. Cultures on Czapek-Dox solution with 3% glucose produce in some cases a yellow color in the solution near the top; other strains produce no color.

Penicillium variabile Sopp (4) One of the *P. purpurogenum* series.

Penicillium viridicatum Westling, series (6, wheat field.) Cultures somewhat zonate above and yellow to reddish in reverse.

***Penicillium* spp.** (about 30, 1%) A few mixed cultures with an overgrowth of *Rhizopus*, *Trichoderma*, etc., were discarded. They were probably for the most part common *Penicillia*.

Scopulariopsis brevicaulis (Sacc.) Bainier (5, four of which were extra.) This fungus is known to be common in Manitoba upon old hay and spoiled silage, but it was not found often in cultures from the soil, partly because it starts somewhat more slowly than many other soil fungi. The spores are rough and comparatively large.

Scopulariopsis rufulus Bainier. (1) Culture coffee-brown, darker below; spores $5-7 \times 5 \mu$, faintly tuberculate.

Sporotrichum roseum Link (9, six of which were extra isolations.) As Miss Dale (5, p. 53) states, this fungus is a variable and slowly growing form; it is apt to be swamped by other fungi before it develops noticeably. Colonies white to reddish or purplish, dark purplish brown below. Spores small, $3-4 \times 2-3 \mu$.

Trichoderma album Preuss (21, nearly 1%). This white *Trichoderma* commonly makes very little growth on Czapek's agar, but some strains placed with this species grow upon it readily. Although the fungus remains white or whitish upon the agar media tested, abundant green or greenish masses of spores are formed by some strains upon sterilized leaves or sticks of *Acer negundo*.

Trichoderma glaucum Abbott (4) Occasionally one finds a *Trichoderma* which resembles *T. album* in appearance, but becomes yellowish to pale greenish, and has larger spores (about $4 \times 3 \mu$) than *T. album*.

Trichoderma koningi Oud. (70, 3%). A somewhat broad interpretation of this species has been used to include the more or less floccose green *Trichodermas* with oval spores.

Trichoderma lignorum (Tode) Harz (22, 1%). This fungus is fairly common in soils. It produces a thin growth of mycelium on Czapek's agar, with tufts which become green and bear many small spores $2\frac{1}{2}-4 \mu$, roundish or slightly oval. The parasitism of this fungus on other fungi is discussed below.

Verticillium ?glaucum Bonord. (6, two of which were extra isolations.) The colony characters are as described by Waksman (23, p. 136), being yellow-green above, olive green below. Spores are produced very abundantly, and are $3-7 \times 2-3 \mu$. Conidiophores branched singly or in whorls, producing heads of spores. *V. glaucum* is described as having globular spores; this fungus may be nearer to *V. alboatrum* Reinke and Berth.

Verticillium ?terrestre (Link) Sacc. (6). A pure white floccose growth is produced, with more or less verticillately branched conidiophores bearing heads of small spores $3-5 \times 2-3 \mu$.

B. Dematiaceae

Alternaria tenuis Nees, group (7) Spores about $28-45 \times 10-12 \mu$.

Alternaria spp. (31, more than 1%). Various forms of *Alternaria* appear commonly in cultures from soil.

Botryotrichum atrogriseum van Beyma (2) Cultures dark gray on surface, nearly black below. The original cultures showed spores from $13-22 \mu$ in diameter, but in later transfers they are only $11-16 \mu$.

Botryotrichum piluliferum Sacc. and March. (2, garden.) Spores $9-14 \mu$, globose, smooth, colony dark gray above, yellowish-brown below.

Cladosporium herbarum (Pers.) Link (69, nearly 3%) Forms resembling *C. epiphyllum* and *Hormodendron cladosporioides* were found. The spores are sometimes smooth, sometimes rough; commonly one-celled, but often with two or three cells. A common and variable fungus in the soil. Fungi belonging to other species of *Hormodendron* are sometimes found, but were not identified.

Coniosporium arundinis (Corda) Sacc. (1) The spores are 5–8 μ wide in one diameter, about 4 μ in side view, marked with a hyaline line.

Helminthosporium sativum Pam., King, and Bakke (7, four of which were extra, all from wheat fields.) Identifications made or verified by Dr. J. E. Machacek. There is some variation in the appearance of the different cultures, but the spores are similar in all cases, 30–80 \times 17–27 μ , the larger spores mostly five to seven-celled, olive green to black. The parasitism of this fungus was demonstrated as described below.

Mycogone nigra Jensen, probably not the same as *Monotospora nigra* Morgan. (18, principally from wheat field.) This fungus which Jensen (13) obtained from soil, is probably the same as the fungus referred doubtfully to *Basisporium gallarum* by Miss Dale (4, 5) and Waksman (23). The cultures produce a typical growth at first yellow then nearly black, the reverse becoming very dark and zonate. The spores have a small pale basal cell.

Stemphylium spp. (5)

Torula convoluta Harz (3, garden.) A dark fungus which produces spores 4–6 \times 4–5 μ , and these collect in small heads.

Trichocladium asperum Harz (2, garden.) Spores two-celled, the upper cell larger.

C. Other Fungi Imperfecti

Colletotrichum sp.? (4) Cultures first pinkish, with an abundant, thin, moist layer of spores, then becoming black with sclerotium-like masses of mycelia, which sometimes bear small setae. Spores 10–12½ \times 3–4 μ , sometimes smaller, often one-septate. The fungus somewhat resembles *C. atramentarium*, but is probably not that species.

Coniothyrium spp. (14) Three or more species of *Coniothyrium* have been isolated. The dark spores in some cases are small, 3–5 \times 2 μ , or roundish, 3½–4 μ ; in one isolation the spores are 5–8 \times 3–4 μ . These fungi no doubt represent stages of *Pyrenomyces* which grow on old vascular plants or their parts. It would be difficult or impossible to apply specific names with any meaning to fungi such as *Phoma* or *Coniothyrium* without knowing the hosts.

Cytospora sp.? (1, from river bank.) Stromatic masses of pycnidia with allantoid, pale olive colored spores 3–5 \times 1 μ . This fungus perhaps developed in nature upon some twig.

***Pestalozzia* sp.** (1, forest soil.) A fungus belonging to the section *quadriloculare*, spores $18-22 \times 6-7 \mu$, with three setae on the end about as long as the spore; two centre cells brown, end cells hyaline. Probably entered the soil from some part of a vascular plant.

***Phoma* spp.** (29, 1%) The *Phoma* stages of *Pyrenomyces* which probably grow in nature upon higher plants or their remains are fairly frequently isolated from soil. Pycnidia are often produced abundantly in cultures, and spores are found in some cases as small as $2-4 \times 1\frac{1}{2} \mu$, in others as large as $14-19 \times 2\frac{1}{2}-3\frac{1}{2} \mu$. Some of these may represent species of *Phyllosticta*.

Undetermined fungi (about 300, 12%) In making large numbers of isolations from soil, one inevitably fails to determine every fungus. About 130 cultures of fungi (*Fusaria*, *Trichoderma*, etc.) were mixed, lost before identification, etc.; these were probably for the most part species already included on previous pages. There still remain, however, about 170 cultures, belonging to perhaps a score or more of species, for which names could not be obtained. Some of these fungi have not been induced to form spores; others produce only microconidia; others belong to very difficult groups. Some of these fungi perhaps represent haploid phases of *Hymenomyces* or *Pyrenomyces*, which will not develop perfect stages without mating with the proper haploid of another sex, and there are few clues.

Cultures have been kept of most of the species, known and unknown, and further work may illuminate some of them more clearly.

SUMMARY OF FUNGI ISOLATED

Genus	Number of entries	Number of isolations	Genus	Number of entries	Number of isolations
<i>Absidia</i>	4	15	<i>Monilia</i>	2	3
<i>Mortierella</i>	1	2	<i>Penicillium</i>	25	1,404
<i>Mucor</i>	6	29	<i>Scopulariopsis</i>	2	6
<i>Rhizopus</i>	2	76	<i>Sporotrichum</i>	1	9
<i>Syncephalastrum</i>	1	12	<i>Trichoderma</i>	4	117
<i>Zygorhynchus</i>	3	3	<i>Verticillium</i>	2	12
<i>Chaetomium</i>	2	7	<i>Alternaria</i>	2	38
<i>Fimetaria</i>	1	1	<i>Botryotrichum</i>	2	4
<i>Gymnoascus</i>	1	1	<i>Cladosporium</i>	1	69
<i>Saccharomyces</i>	1	9	<i>Coniosporium</i>	1	1
<i>Sporormia</i>	2	4	<i>Helminthosporium</i>	1	7
<i>Rhizoctonia</i>	1	1	<i>Mycogone</i>	1	18
<i>Ustilago</i>	1	1	<i>Stemphylium</i>	1	5
<i>Acrostalagmus</i>	1	5	<i>Torula</i>	1	3
<i>Aspergillus</i>	7	50	<i>Trichocladium</i>	1	2
<i>Botrytis</i>	2	6	<i>Colletotrichum</i>	1	4
<i>Cephalosporium</i>	4	47	<i>Coniothyrium</i>	1	14
<i>Cylindrocarpum</i>	4	24	<i>Cytospora</i>	1	1
<i>Dactylium</i>	1	1	<i>Pestalozzia</i>	1	1
<i>Fusarium</i>	17	128	<i>Phoma</i>	1	29
<i>Geotrichum</i>	1	2	Unknown	—	300
<i>Ghiocladium</i>	4	53	Totals:		
<i>Hymenula</i>	1	13	44 genera known	121	2,565
<i>Metarrhizium</i>	1	28	entries		cultures

It may be noted that in ten cases the genus was determined, but the one or more species found were undetermined. There are also a few varieties which are not counted in the entries for species. The number of species actually named is 100 (exclusive of varieties) and a few of these specific names are questionable. The total number of distinct species of fungi actually found in the soil is in the neighborhood of 150.

The Numbers of Fungi in Surface Soils of Manitoba

The Manitoba soils supporting plant life gave counts of fungi varying from 18,000 to 350,000 per gram. A sample taken in winter from below the average summer water line on the bank of the Red river showed only 133 and two others from above that line gave 1,700 and 3,000 respectively. The highest counts were obtained from forest soils, although an alfalfa field sampled in January gave 246,000 and in June 195,000 molds. Details of the relative numbers and kinds of fungi in the various types of soil investigated in a preliminary way are not presented since the significant differences are not clear.

The most extensive study thus far made was conducted with soil from two adjacent wheat plots. From 1919 to 1931 these two plots were not distinct, but constituted a plot run on a three-year rotation of wheat, wheat, fallow, without fertilizer or barnyard manure. In 1932 the plot was divided, half being left unfertilized, and half fertilized at seeding with ammonium phosphate containing 10% nitrogen and 48% phosphorus pentoxide, at the rate of 45 lb. per acre in one application. Twenty samples were taken from each of the two plots which differed, so far as known, only in the addition of fertilizer to one of them during the course of the tests. Table I presents the counts, and indicates clearly that the addition of fertilizer gives an immediate and consistent increase in population of fungi.

The irregular fluctuations in numbers of fungi in a soil are not easily explained. Soil temperature and moisture no doubt play a part; it would seem that the number of fungi rises soon after moisture increases, and *vice versa*; with higher soil temperatures, the fungi may increase. It will be noted that the numbers of fungi fluctuate in a similar way in both the fertilized and unfertilized plot, the former numbers remaining higher.

Newton (16), Erdman (7), Brierley (2, 3) and others have discussed these seasonal fluctuations in numbers of fungi in soil. Plate counts at least give evidence of the sensitiveness of the soil population to environmental changes.

Although there is definite fluctuation in numbers of fungi in soil, little evidence was found that the cold of a long Manitoba winter reduces the number of viable spores in soil. Thus a garden plot gave counts of 74,000 fungi per gram in December, and 50,000 in late June; an orchard soil 91,000 in January and 87,000 in June; and alfalfa field 246,000 in January and 195,000 in June. Erdman (7) found a maximum count of molds in an Iowa soil in January.

The numbers of fungi in Manitoba soils are similar to those found in other soils in North America; indeed they are higher than many recorded by Waksman (25) but not as high as Miss Todd (22) reports for Oklahoma.

TABLE I
NUMBERS OF FUNGI DURING SEASON IN PLOTS FERTILIZED* AND UNFERTILIZED

Date sample taken 1932	Moisture, % (dry basis)	Temperature,** ° F.	Numbers of fungi per gram of soil	
			Unfertilized	Fertilized*
April 22	42.9		46,000	44,000
April 29	43.7	36.0	51,000	39,000
May 6†	37.0	41.9	78,000	86,000
May 13	35.5	52.9	80,000	138,000
May 20	34.6	54.6	62,000	85,000
May 27	30.1	54.1	61,000	70,000
June 2	29.0	57.1	37,000	42,000
June 10	23.3	67.4	91,000	190,000
June 20	37.6	67.7	107,000	288,000
June 25	30.9	65.3	102,000	127,000
July 4	29.8	63.1	152,000	212,000
July 14	27.0	65.4	65,000	133,000
July 25	22.1	71.9	98,000	133,000
Aug. 4††	17.7	68.2	70,000	129,000
Aug. 16	13.9	68.9	25,000	58,000
Aug. 31	39.1	71.2	18,000	47,000
Sept. 13	22.2	—	127,000	96,000
Sept. 29	34.4	50.5	68,000	100,000
Oct. 13	34.4	43.6	48,000	78,000
Nov. 11	43.4	32.0§	87,000	60,000
Average of 18 tests after fertilizer applied			76,400	115,100

*Ammonium phosphate drilled in with seed.

**Average of readings taken twice daily at 8 a.m. and 4 p.m. covering the period since previous sampling. The thermometer was in a perforated wooden case placed in the ground so that the mercury bulb extended 3 in. below the surface.

†Plot seeded and fertilized May 2. First sample after fertilizing.

††Crop harvested August 4.

§Temperature at final sampling.

Parasitic Fungi Isolated from Soil

Plant pathologists have found that a considerable number of parasitic fungi remain alive in soils, sometimes for years. Fungi causing root rots of cereals, flax, and other cultivated plants are particularly troublesome and persistent in soils. Species of *Fusarium* are important plant pathogens capable of saprophytic existence in soil, and *Fusaria* are obtained frequently from isolations made by soil microbiologists. LeClerc (14) reports that 23% of the fungi isolated from Colorado soils were *Fusaria*; Gilman and Abbott (9) list 27 species of *Fusarium* as having been isolated from soil. These fungi are common also in Manitoba soils, and *F. culmorum*, known to cause root rots, is one of the commonest. A culture of *F. culmorum* isolated from a wheat field was found to be parasitic to wheat, as described in the next section.

The methods of soil microbiology have been subject to some criticism because they have failed to demonstrate the occurrence in soil of *Helminthosporium sativum*. This fungus appeared, however, several times in isolations from wheat fields, and was found to be parasitic to wheat.

Rhizoctonia solani, another fungus parasitic to many plants, was isolated once from Manitoba soil. This fungus does not produce spores in its ordinary vegetative growth, and the *Corticium* stage seldom is found in Manitoba, so that *Rhizoctonia* is not likely to be isolated as frequently as its prevalence in soil might warrant.

Fungi parasitic to aerial parts of plants sometimes may find their way into a soil sample: thus the *Cylospora* and some of the isolations of *Phoma* and *Coniothyrium* perhaps represent the imperfect stages of fungi more or less parasitic to plants. *Botrytis cinerea* also may develop parasitically upon higher plants.

The methods of soil microbiology yield data of value regarding the parasitic fungi in a soil, and these data have some quantitative value. For example, about 7000 colonies developed from 40 isolations from the wheat plots; seven of these were found to be *Helminthosporium sativum*. We may conclude that *H. sativum* exists in this soil in a proportion of the order of 7 : 7000 total. The average number of fungi found in the wheat field was 90,000 per gram of surface soil: the calculation indicates that each gram of a soil cropped to wheat for some time in Manitoba may contain 90 viable spores or bits of mycelium of *H. sativum*. Henry (12) has studied this fungus in Alberta soils.

Soil Fungi Parasitic upon other Fungi

When one considers the possible prevalence in the soil of fungi parasitic to crop plants, it becomes evident that nature must strike a balance in some way, or crops could not be grown. Sanford and Broadfoot (18) and Henry (11) have discussed this problem as pertaining to soils in western Canada.

During July, 1932, the writers made isolations from a soil which was used as a "disease garden" to test resistance of barley varieties to soil fungi. Plots had been inoculated heavily with pathogenic *Fusaria* and *Helminthosporium*, and adjacent plots left as checks. The barley showed no obvious difference on the two plots. Soil isolations were made, and colonies of *Fusarium culmorum* started in considerable numbers upon plates from the inoculated soil, but it was striking to note the rapidity with which *Trichoderma lignorum* overwhelmed and destroyed these colonies of *Fusaria* on petri dishes. *Trichoderma* previously had been considered as merely a rapidly spreading mould; it now appeared as a beneficial parasite. Before tests were made to corroborate the observation, Weindling (26) published a paper which shows clearly that *T. lignorum* parasitizes certain soil fungi (*Rhizoctonia solani*, *Phytophthora parasitica*, *Pythium* spp., *Rhizopus* spp., and *Sclerotium rolfsii*).

A test of the efficacy of *T. lignorum* in preventing injury by pathogenic fungi to wheat in soil was undertaken in the following manner. Pots of soil

were sterilized at 15 lb. pressure for three hours, and to them were added (1) heavy inoculation of *Fusarium culmorum*, soil isolation, growing on oat hulls plus peptone; (2) similar inoculation with *Helminthosporium sativum*, soil isolation; (3) *Trichoderma lignorum* alone; (4) *F. culmorum* plus *T. lignorum*; (5) *H. sativum* plus *T. lignorum*; (6) all three fungi together; (7) check, no fungi added. Two pots of each were used; on November 7, 1932, ten seeds of Mindum wheat, after surface sterilization, were planted in each pot. As soon as the plants emerged it became evident that the *Fusarium* and *Helminthosporium* were injurious alone, and that *Trichoderma* alone caused no noticeable injury to the plants, and in combination with the other two fungi, rendered them harmless. Figs. 1 and 2 illustrate the condition of the plants on December 5, 1932.

It is certain that *Trichoderma lignorum* is one fungus which plays an important part in reducing the danger to crops from pathogenic fungi in the soil. *T. lignorum* was found in surface soils of Manitoba to the extent of nearly

1% of the fungi present; this would indicate that each gram of surface soil harbors from 200 to 3000 viable spores or bits of mycelium of this *Trichoderma*. It is likely that *T. koningi*, certain *Penicillia*, and other organisms also may reduce the pathogenic action of soil fungi. *T. lignorum* and other species undoubtedly parasitize saprophytic fungi as well, and in turn presumably are held in check by other organisms, all of which contribute to the "balance" of the micro-organisms in a soil.

Another type of fungus-parasitism is shown by *Dactylium dendroides*, isolated once from a forest soil. This fungus is found occasionally in Manitoba parasitizing larger *Hymenomyces* in the woods.

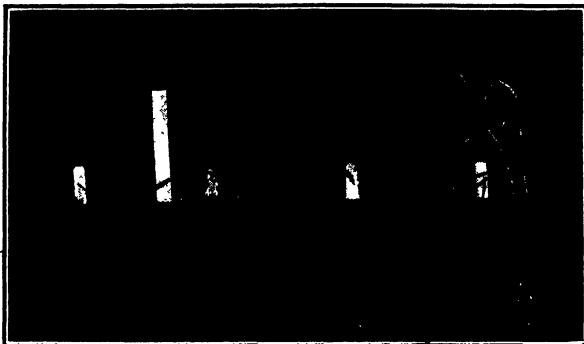


FIG. 1. From left to right: *Helminthosporium sativum* (soil isolation) added to sterilized soil. Second pot, *H. sativum* and *Trichoderma lignorum*. Third pot, *H. sativum*, *Fusarium culmorum* and *Trichoderma*. Fourth pot, check.



FIG. 2. From left to right: *Fusarium culmorum* added to sterilized soil. Second pot, *F. culmorum* and *Trichoderma lignorum*. Third pot, *T. lignorum* only. Fourth pot, check.

"Soil Fungi" Previously Found in Manitoba

Manitoba has been surveyed relatively carefully for the parasitic and saprophytic fungi found on plants or plant parts and on the ground in the fields and forests. This survey, covering about 2000 species of fungi has been published (1). When the fungus-flora of the soil is compared with that found above the soil, it becomes evident that the large majority of the soil fungi are not encountered in an ordinary mycological investigation of an area. The following only, of the fungi herein recorded from Manitoba soil, were included in the book (1) on the fungi of Manitoba:—

Mucor racemosus, common on dung.

Rhizopus nigricans, common on various substrata.

Fimetaria fimicola, on dung.

Gymnoascus reessii, occasional on dung.

Aspergillus fumigatus, on mouldy plant parts.

Aspergillus niger, sometimes appears on old plants, etc.

Botrytis cinerea, parasitizes various plants.

Dactylium dendroides, found on larger fungi.

Fusarium coeruleum, causes rot of potato tubers.

Fusarium culmorum, parasitizes cereals.

Fusarium oxysporum, in wilted potatoes.

Scopulariopsis brevicaulis, a variety on cheese.

Rhizoctonia solani, on roots of potatoes, etc.

Trichoderma lignorum, common on wood and bark.

Cladosporium herbarum, very common on old plant parts.

Helminthosporium sativum, injurious to cereal roots.

Many of the fungi found in soil, of course, would be obtained by making isolations from old leaves, debris, etc.

Does the Isolation Technique of the Soil Microbiologist give a true Picture of the Soil Fungi?

The methods of counting soil fungi have been standardized fairly well (3) but there are various objections to these methods, as expressed by Brierley *et al.* (2, 3), Thom (21), and others. Attempts have been made to obtain other perspectives by Winogradsky's direct microscopic examination of soil, and by Chododny's technique in which microscope slides are placed in the unmolested soil for one to three weeks before examination. Isolation methods, however, have the advantage of yielding cultures which usually are determinable.

Since spores of fungi fall upon the soil from all sources, one might expect to isolate from soil almost any fungus that grows readily on common agar media. It must be remembered, however, that the true soil fungi, *i.e.*, those which live and reproduce in the soil, will be isolated nearly always in larger numbers than those fungi which have grown elsewhere. Furthermore, enough is known of the common fungi to make it possible usually to estimate the

source of the fungus once its name is determined. If a fungus is present to the extent of 1% or more of the total number isolated, it is safe generally to conclude that it is an active soil fungus.

Many important soil fungi however, are rarely or never obtained by the ordinary isolation technique. The mycelium of *Hymenomycetes*, for example, unquestionably plays an important part in the disintegration of organic matter in soils, particularly forest soils, and spores of these fungi fall in great numbers upon the ground. Since the larger fungi are often heterothallic, however, cultures obtained seldom are identifiable even to group. Isolation technique needs to be supplemented by field study to gain a proper idea of the soil fungi.

Fungi which grow slowly on culture media usually are swamped on ordinary isolation plates, but a number of cultures of such fungi may be obtained by examination of plates from higher dilutions held longer. Possibly a few fungi of some importance in soil will not grow on common agar media.

An analysis of the data presented in this paper indicates that the isolation methods used give a picture of the activities of soil fungi which must be true in many respects. *Mucorales*, *Penicillia*, *Trichoderma* and other saprophytes known to be important in breaking down organic material have been obtained abundantly. Smaller counts of the parasitic fungi known to exist in field soils were found. *Trichoderma* is important in preventing the parasites from dominating the soil. A number of common fungi likely to be present accidentally in surface soils have been found there in relatively small numbers. Isolation methods give consistent counts showing that fungi are more common in forest than in field soils, and that they may increase when fertilizer is added. They show that the fungi are only dormant in a cold winter.

Care must be taken in soil isolation work to preclude laboratory contaminations in the plates counted. It is almost inevitable that an occasional spore from the air may enter and the resulting colony be counted as a soil fungus. This source of error tends to raise the count of *Penicillia* in particular, but can be eliminated to practical insignificance.

Variation in Soil Fungi

After examining a few thousand cultures of fungi, one is impressed by the variation many of them show. These may be classified as morphological variations, represented by slight differences in size, shape, etc., of spores, in characters of conidiophores, or in type of growth on culture media; and physiological variations shown in color changes produced in the media, or variations in odors, etc. For example, *Penicillium terrestre* often has a very characteristic fruity odor; but more often it is lacking in cultures morphologically indistinguishable. While some of these variations might be worthy of variety or form names, in many cases there are intergradations which would make a varietal name useless or confusing. Certain groups of fungi, such as *Trichodermas* and *Cephalosporiums*, need critical revision; but most soil fungi can be identified, if one allows something for the variations that occur.

The Distribution of Soil Fungi

Many of the fungi found in Manitoba soils have a very wide distribution over the world. Miss Dale (4, 5) found 57 species of fungi in soil in England, more than one-third of which have been found in Manitoba soil. Gilman and Abbott (9) give distribution records from which it is evident that many soil fungi have a wide distribution. Brierley (2) considers that *Aspergilli* may be more common in warmer temperate regions, and Miss Todd (22) reports, as mentioned above, high counts of *A. niger* in Oklahoma. *Aspergilli* are not very abundant in Manitoba soils.

Critical comparisons of the distribution of soil fungi are not possible until more data are available. LeClerc (14) has analyzed 7000 isolations from Colorado soils, 6880 of which he includes under 31 names. He also found *Penicillia* to be the commonest soil fungi, but found a higher proportion of *Aspergilli* than have been found in Manitoba.

There are definite differences in the fungus-flora of distinct soil types, or from the same type of soil cropped to different plants, but the work here reported has not progressed far enough to make clear the significant differences, nor to explain the variations in number or types of fungi found in the soil during the four seasons. It is believed, however, that sufficient knowledge of the fungi in surface soils in Manitoba has been gained to make possible further results of practical value from an intensive study of certain phases of their activity.

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THE EFFECT OF MECHANICAL SEED INJURY ON THE DEVELOPMENT OF FOOT ROT IN CEREALS¹

BY J. E. MACHACEK² AND F. J. GREANEY³

Abstract

Greenhouse and field experiments have shown that the use of mechanically injured seed promotes the development of seedling blight and foot rot caused by *Fusarium culmorum* in cereals, thereby retarding the growth of the plants and decreasing yield.

The presence of a large proportion of broken or bruised kernels in seed grain seriously reduces its market value. A secondary loss, caused by invasion of seed grain by fungi after the seed is sown, also occurs but very few experimental data regarding this loss are available.

It is known that fruits and vegetables are protected from the invasion of many rot-inducing micro-organisms by cuticular and epidermal layers, or waxy exudations known as "bloom". Seeds of the *Gramineae* are similarly protected. Brown (1) observed that a semipermeable membrane covers the seed, making it somewhat impermeable to toxic salts. It has been found by other investigators that the permeability of the testa is increased when the tannin content of the testa is low (11, 12), when the testa is undeveloped owing to drought (3), and when the seed is unripe or damaged (6). Nobbe (9), according to Hurd (5), found that machine-threshing injured the seed coat sufficiently to permit chemical injury in the seed after treatment with copper sulphate. Walldén (13), according to Hurd (5), confirmed Nobbe's findings, and was able to correlate the degree of chemical injury with the amount of damage to the seed coat. Pugh and others (10) found that the testa of wheat kernels becomes increasingly resistant to penetration by *Gibberella saubinetii* (Mont.) Sacc. as the grain matures. Hurd (5) found that chemical or mechanical seed injury predisposes the seed to attack by saprophytic fungi. Walldén (13) also reported marked susceptibility to moulds in mechanically injured seed when the grain was stored under improper conditions.

The data presented here show that mechanical injury predisposes seed grain to infection by fungi, particularly *Fusarium culmorum* (W. G. Sm.) Sacc., and consequently, in infested soil, seedlings arising from injured seed are more frequently diseased than seedlings from uninjured seed.

Materials and Methods

Several varieties of wheat and a hull-less variety of oats and of barley were used in these experiments. The seed coat was injured by one of three methods: (a) puncturing slightly the embryo end, (b) clipping off a small portion of the "brush" end, or (c) scarifying the seed lightly with coarse sandpaper. The effect of each type of injury was determined by planting the seed in autoclaved soil to which a quantity of *F. culmorum* inoculum was added. The inoculum consisted of a fresh culture of the fungus grown on an autoclaved, ground oat-hull medium.

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Experimental Results

Greenhouse Experiments

In the majority of the greenhouse experiments only punctured or clipped seed was used. The inoculum (one part inoculum to nine parts soil, by volume) was added to autoclaved soil, and both were mixed by means of a drum mixing machine. In each test four six-inch pots, each containing 25 seeds planted at a depth of 1 in., were used for each type of seed injury. Checks with uninfested autoclaved soil were used. The pots were kept at approximately 20° C. and watered daily.

Notes on seedling emergence and disease prevalence were taken two weeks after planting. It was observed that wherever injured seed, as compared with uninjured seed, was planted in infested soil, reduced emergence and increased foot rot occurred. In calculating the percentages of foot-rotted plants only the emergent seedlings were considered. The results are illustrated graphically in Figs. 1 and 2, and summarized in Tables I and II.

Field Experiments

These experiments were designed to determine the effect of mechanical seed injury on foot-rot development under field conditions. Healthy and scarified seed of Marquis and Mindum wheats were used. The complete field experiment in 1932 consisted of a series

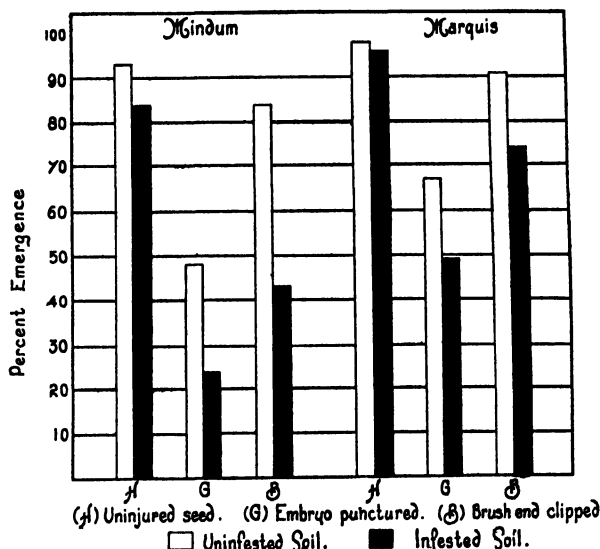


FIG. 1. The effect of mechanical seed injury on the incidence and severity of *Fusarium culmorum* infection in the greenhouse. A. Seedling emergence.

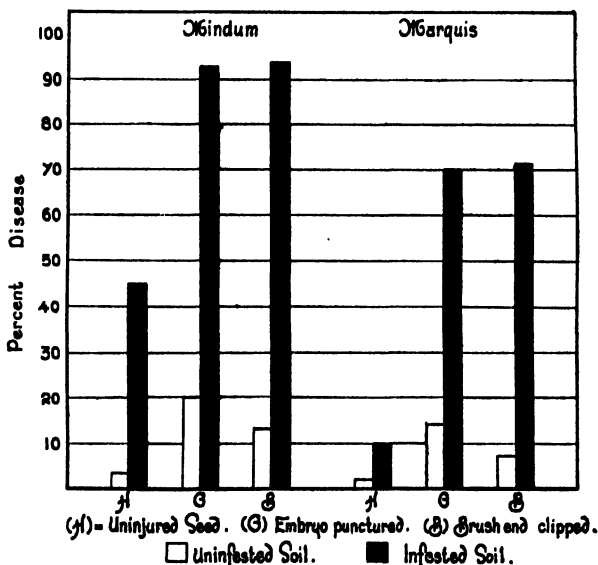


FIG. 2. The effect of mechanical seed injury on the incidence and severity of *Fusarium culmorum* infection in the greenhouse. B. Percentage disease in emergent seedlings.

of eight blocks, each block composed of eight plots. Each plot consisted of two rod-rows, one in which the seed was spaced, 100 to the row, and the other containing 15 gm. of Marquis or 18 gm. of Mindum seed sown by hand and distributed evenly in the row. The inoculum, 600 cc. (1 : 9 mixture, as used in the greenhouse, but incubated for two weeks) per rod-row, was distributed evenly at seed level through each row to be infested. There was complete randomization of varieties (Marquis and Mindum), seed treatments (injured and uninjured), and soil treatments (infested and uninfested) within each block.

TABLE I

THE EFFECT OF MECHANICAL SEED INJURY ON THE AVERAGE EMERGENCE OF SOME CEREAL VARIETIES GROWN IN THE GREENHOUSE IN UNINFESTED SOIL AND SOIL INFESTED WITH *Fusarium culmorum*

Variety	Percentage of emergence			Mean
	Uninjured seed	Embryo punctured	Brush end clipped	
Marquis	97.0	57.5	82.2	78.9
Mindum	88.7	38.0	63.2	60.0
Pen. x Ma. 729	63.0	38.5	67.0	56.1
H-44 x Ma. 586	78.0	49.5	83.0	70.1
Laurel (oats)	70.7	64.0	69.0	67.9
Guy Mayle (barley)	69.0	46.7	67.0	60.9
Mean	79.7	49.2	71.3	

TABLE II

THE EFFECT OF MECHANICAL SEED INJURY ON SEEDLING EMERGENCE AND SEVERITY OF *Fusarium culmorum* FOOT ROT IN THE GREENHOUSE

Treatment	Uninjured seed	Embryo punctured	Brush end clipped	Mean
A. Average emergence in six cereal varieties, per cent				
Uninfested soil	95.1	71.8	91.2	82.7
Infested soil	63.3	26.7	51.4	47.1
Mean	79.7	49.2	71.3	
B. Percentage foot rot in emergent seedlings				
Uninfested soil	12.9	14.3	13.4	13.5
Infested soil	41.6	84.6	78.0	68.1
Mean	27.2	49.4	45.7	

Approximately ten days before harvest, the plants in the spaced rows were pulled and examined individually for the purpose of obtaining a disease rating based on the presence or absence of basal lesions indicating foot rot.

TABLE III

THE EFFECT OF MECHANICAL SEED INJURY ON THE INCIDENCE OF *Fusarium culmorum*
FOOT ROT OF WHEAT IN FIELD PLOTS

A. Soil treatments and disease rating

Treatment	Disease rating		Mean	Standard error 0.90
	Uninjured seed	Sand-papered seed		
Uninfested soil	66.1	68.2	67.2	
Infested soil	72.1	77.4	74.7	
Mean	69.1	72.8		
	Standard error 0.71			

B. Varieties and disease rating

Variety	Disease rating		Mean	Standard error 1.26
	Uninjured seed	Sand-papered seed		
Marquis	65.8	69.3	67.6	
Mindum	72.4	76.4	74.4	
Mean	69.1	72.8		
	Standard error 0.71			

C. Soil treatments and yield

Treatment	Yield in bushels		Mean	Standard error 0.96
	Uninjured seed	Sand-papered seed		
Uninfested soil	38.9	35.7	37.3	
Infested soil	33.4	29.7	31.5	
Mean	36.1	32.7		
	Standard error 1.07			

D. Varieties and yield

Variety	Yield in bushels		Mean	Standard error 1.91
	Uninjured seed	Sand-papered seed		
Marquis	28.9	25.0	27.0	
Mindum	43.4	40.5	41.9	
Mean	36.1	32.7		
	Standard error 1.07			

From the data so obtained a disease rating was calculated for each plot. A modification of the method described by McKinney (8) was used. The second row in each plot furnished the yield data for the plot.

The analysis of variance method, as devised by Fisher (4), was used in analyzing the disease-rating and yield data. The effect of soil treatments and of varieties on disease rating in plants grown from injured and uninjured seed and the effect of the same factors on yields from injured and uninjured seed plots are shown in Table III. It will be observed that significant increases in disease rating and significant decreases in yield resulted from seed injury. The wheat variety Mindum appeared more susceptible to *F. culmorum* foot rot than the variety Marquis. In the final analysis of the experimental results, disease ratings and yields of individual plots were correlated, and a highly significant negative coefficient (-0.5129) was obtained.

Discussion

The mechanical injury of seed appears to be an important factor in the development of pathologic conditions other than the foot rot caused by *F. culmorum*. The investigations of Hurd (5) and Walldén (13) show that mechanically injured seed is particularly susceptible to invasion by saprophytic fungi. Hurd found that mechanical injury lowers seed vitality, although germination may be unimpaired. If favorable conditions are present, the injured seed, though its initial germination is good, soon becomes invaded by saprophytic fungi, the invasion resulting in distortion and stunting of the seedlings, and often in lesioning of the coleoptile and primary roots. Hurd believes that the seed testa protects the seed from fungous invasion, and that, if the testa is broken, the nutrients contained in the endosperm become readily available to these fungi, resulting in their luxuriant growth when conditions of temperature and moisture permit. Consequently, according to her findings, mechanical seed injury, as well as other factors that lower vitality of seed grain, predisposes the seed to invasion by fungi.

It is probable that the same host-parasite relationship exists in the development of *F. culmorum* foot rot in cereals. Through mechanical injury of the seed the endospermic nutrients become readily available to the fungus, thereby promoting its growth, but at the expense of seed and seedling. That this theory is at least partly correct is shown by the examination of the greenhouse experimental data. A considerable number of seedlings failed to reach the soil surface when injured seed was planted in infested soil, whereas uninjured seed produced seedlings that, under similar conditions, were able to emerge from the soil in large numbers. The possible increased growth of the fungus, in the case of the injured seed, is directly reflected in the rapid development of pre-emergence blight. It would therefore seem probable that the use of uninjured seed would be advantageous to the farmer in his attempt to produce a disease-free crop.

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ON THE SPECIFIC HEATS OF TUNGSTEN, MOLYBDENUM, AND COPPER¹

By H. L. BRONSON², H. M. CHISHOLM³ and S. M. DOCKERTY³

Abstract

This paper contains the results of a long series of determinations of the specific heats of tungsten, molybdenum, and copper from -20° to 500° C.

A new type of all-copper adiabatic calorimeter has been designed and used. The complete elimination of water from the calorimeter removed several sources of error and resulted in increased reliability and accuracy.

Two entirely different methods were used in determining the specific heats. The usual "method of mixtures" was used to determine the mean specific heat for a large temperature change and was applied to all three metals over the entire range of temperature. The specific heat of copper was also determined for 5- or 10-degree intervals from -5° to 110° C. by heating the calorimeter electrically.

It has been quite definitely shown that the specific heats of these metals over a temperature range as large as 0° to 500° C. cannot be expressed as a linear function of the temperature. An equation of the form $C_p = A + BT - C/T^2$ was arrived at from theoretical considerations and the constants determined empirically with the following results:—

$$\text{Tungsten} \dots\dots\dots C_p = 0.03199 + 0.00000328T - 129/T^2$$

$$\text{Molybdenum} \dots\dots\dots C_p = 0.06069 + 0.0000120T - 361/T^2$$

$$\text{Copper} \dots\dots\dots C_p = 0.09292 + 0.0000136T - 452/T^2$$

where the unit of heat is the 20-degree calorie and T is absolute temperature. The average deviation of the individual determinations from the values calculated by these equations was only about 0.1%.

As a matter of convenience and for purposes of comparison, linear equations applicable over smaller ranges of temperature have also been given.

Introduction

Many problems both theoretical and practical require for their solution reliable and accurate values of the specific heats of the metals. An examination of the specific heat data given in the International Critical Tables (I.C.T.) shows that such values are not available. Most of the data are old and there are wide discrepancies among the results of different observers. Unfortunately, however, wide discrepancies are not confined to the older work. For example, at the time that this investigation was started, two of the most recent and presumably reliable values (7, 9) for the specific heat of tungsten differed by about 30%.

The value obtained for the specific heat of a body by the usual calorimetric methods is the mean value for a considerable change of temperature. The final temperature is most commonly about room temperature and the initial temperature 100° C. or higher. For some of the metals one such mean value over a single temperature range is all that is given in the I.C.T. This is entirely unsatisfactory where even a moderate degree of accuracy is required, since the change in specific heat from 0° to 100° C. may be as much as 5%.

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In the cases of most of the more common metals for which more data are available the I.C.T. combines all the information into "best" values and expresses the specific heat as a linear function of the temperature. It is difficult to estimate the accuracy of the values given but an uncertainty in some cases of as much as 50% in the value given for the temperature coefficient of specific heat would seem quite possible. In fact, two values are given in the case of aluminium which differ by more than this.

The results of the present investigation show very conclusively that the temperature coefficients of specific heat of tungsten, molybdenum, and copper are not constant but decrease with increasing temperature, the average value between 0° and 150° C. being about double that between 150° and 500° C. The *rate of decrease*, however, diminishes rapidly with increasing temperature becoming at 500° less than 2% of its value of 0° C. These facts probably account for many of the discordant results obtained by previous experimenters.

If the change in the temperature coefficient is to be detected and measured, either the mean specific heat for large changes of temperature must be determined with greater accuracy than has been usual in this type of work, or some way must be found of obtaining the actual specific heat at a given temperature, that is, over a small range, for a considerable number of quite different temperatures. Both methods have been employed in the present investigation, with entirely consistent results.

The specific heat of copper is probably known with at least as great accuracy as that of any of the metals. There are, however, discrepancies among the most reliable data which it seems desirable to point out and discuss briefly even though the results of this investigation are anticipated in so doing.

The work of Harper (5) at the Bureau of Standards is a striking exception to the usual practice of measuring the mean specific heat for a large change in temperature. He determined the specific heat of copper for two-degree intervals from 0° to 50° C. and obtained the following linear equation which should be trustworthy for the range given:

$$C_p = 0.0905 + 0.000048t,$$

where C_p is the specific heat at constant pressure in cal./gm.°C. and t is in °C.

A comparison of this equation with another of the same form but for a larger range of temperature shows that the specific heat curve is actually far from linear. Since the "best" values given in the I.C.T. should be as reliable as any available, they were used and the following equation obtained for the range 0° to 500° C.:

$$C_p = 0.0916 + 0.000025t.$$

These two equations give the same value for C_p at about 50° C. and the constant terms differ by about 1%, but the interesting fact is that the temperature coefficient of the first is nearly double that of the second.

In the present investigation the values obtained for the specific heat of copper between 0° and 50° C. differ from those obtained by Harper only by

0.15%. Our experiments, however, were extended to include temperatures up to 500° C. and the temperature coefficient was found to change from about 0.000058 at 0° to 0.000048 at 25°, to 0.000040 at 50°, to 0.000031 at 100°, to 0.000020 at 250°, and to 0.000016 at 500° C., the last value being even smaller than that given in the I.C.T.

It has been quite commonly assumed, for example in the I.C.T., that the relation between specific heat and temperature could be expressed by an equation of the form

$$C_p = A + Bt + Ct^2.$$

In fact, only occasionally has an attempt been made to express experimental results in any form other than a first or second degree equation. That even a second degree equation is quite unsuitable for the purpose is indicated by the above values for the temperature coefficient of copper, which show that the *rate of change* of the temperature coefficient with the temperature decreases rapidly and approaches zero at high temperatures, while it is constant in a second degree equation.

General Method

An adiabatic calorimeter of the Richards' type was used throughout this investigation, but two entirely different methods were employed in the determination of the specific heats. In the first, the specimen under investigation was heated in a suitable electric furnace and allowed to fall directly into the adiabatic calorimeter and its specific heat was calculated as usual in the method of mixtures. Two types of calorimeter were used, the usual one containing water and another where the water was replaced by an all-copper calorimeter. The water equivalent of each calorimeter was determined by heating it electrically and measuring the energy input per degree rise in temperature. The equivalent of the water calorimeter was also calculated in the usual way, using the masses and specific heats of its various parts. By this first method were obtained the *mean* specific heats of tungsten, molybdenum, and copper between room temperature and various other temperatures from -20° to 500° C.

In the second method the solid copper calorimeter was heated electrically from below 0° to above 100° C. The energy supplied per gram per degree rise in temperature was determined for successive small temperature intervals. From this the actual specific heat of copper at a definite temperature was readily calculated. This method was more accurate than the first, but could not be applied to tungsten and molybdenum as sufficient material in suitable form was not available.

An effort was made in this work to discover and eliminate those constant and unsuspected sources of error which have caused such wide discrepancies among the results of different investigators. To this end the apparatus and the methods have been continually altered. The writers have aimed at reliability rather than high precision, but even so it is felt that a higher precision than is usual in this type of work has been attained.

Apparatus and Experimental Methods

The Adiabatic Calorimeter

This consisted essentially of the calorimeter, *C*, Fig. 1, the bomb or "jacket", *B*, and, surrounding *B*, a water bath, which is not shown in the figure. The temperature of the calorimeter was measured by a Beckmann or a platinum thermometer in the outside water bath which was kept in thermal equilibrium with the inside calorimeter.

The equality in temperature between the two was indicated by a sensitive differential thermocouple. Under these circumstances there should be no heat transfer between the calorimeter and surrounding bodies. However, instead of the temperature of the calorimeter remaining constant, there usually occurred a slow change or "creep" in its temperature which necessitated the making of uncertain and time-consuming corrections, occasionally as large as 2%. This "creep" was quite erratic, being sometimes positive

and sometimes negative, and might have been due to a number of causes; e.g., evaporation, stirring, or failure of the differential thermocouple to indicate exact equality of temperature because of non-homogeneous constantan wire.

The calorimeter shown in Fig. 1 was of the usual type, and consisted of a nickel-plated copper beaker of about 500 cc. capacity. Within this was another smaller copper vessel having a cover arranged to close automatically as the specimen entered and thus prevent heat losses by convection. The total mass of copper in this calorimeter was about 200 gm. and from 150 to 400 gm. of water was used in the various experiments. Suitable stirring was obtained by a copper ring which was raised and lowered about 30 times per minute. This stirrer was operated very simply and satisfactorily by means of a worm and wheel attached to one of the motors used in stirring the outside water bath.

The all-copper calorimeter shown in Fig. 2 proved to be a great improvement over the water calorimeter and was used in all the later experiments. With it there was no troublesome water vapor and no stirring. There was the added advantage that the differential thermocouple wires, *T*, were entirely enclosed in a constant-temperature region, instead of having to pass outside the jacket as in Fig. 1. For this reason, probably, the all-copper calorimeters

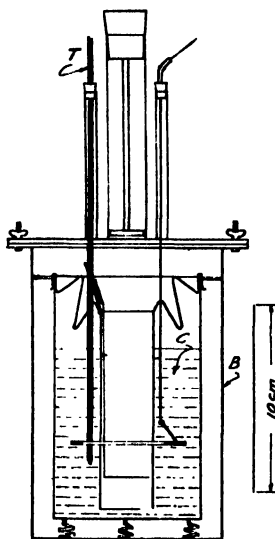


FIG. 1. Water calorimeter and jacket.

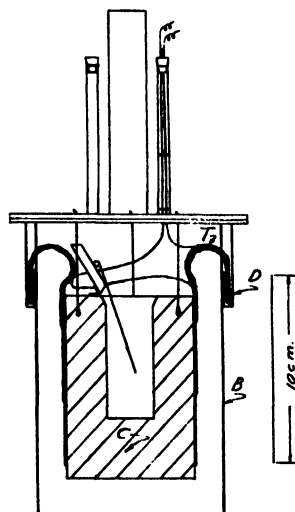


FIG. 2. All-copper calorimeter and jacket.

were practically free from the "creep" which proved so troublesome with the water calorimeters.

Three of these all-copper calorimeters, of masses about 1, 3, and 5 kgm., were made in order that the temperature changes in the various experiments might not be too large to measure with a 5°-range Beckmann thermometer and yet not too small for the desired precision. Each calorimeter had a 2.5-cm. hole drilled as shown and a heavy copper cover arranged to close automatically. Castings for the two larger calorimeters were made by Tallman Brass and Metal Ltd., of Hamilton, Ont., and were said to be of 100% pure copper. The smallest was made from a piece of especially pure 2-in. copper rod supplied by the Halifax Shipyards. The densities of the three were respectively, 8.82, 8.81, and 8.90 gm. per cc. The same jacket was used with all the calorimeters. It was of nickel-plated brass and had a capacity of about 1100 cc.

Water and Oil Baths

The water bath surrounding the jacket was equipped with hot and cold water connections and could be heated electrically as well. After a broken belt had cracked the case of one Beckmann thermometer, the usual stirring paddles with belts and pulleys were replaced by two $\frac{1}{8}$ -h.p. high-speed motors direct-connected by 8-in. extension shafts to the stirring paddles which operated inside short lengths of 2-in. pipe. The motors were fastened to the sides of the tank with shafts nearly vertical. This arrangement was much safer and more convenient than the belts and pulleys usually used. A careful examination with a platinum thermometer showed no variation of over 0.001° in the temperature of the water around the jacket even when it was 25° C. above room temperature.

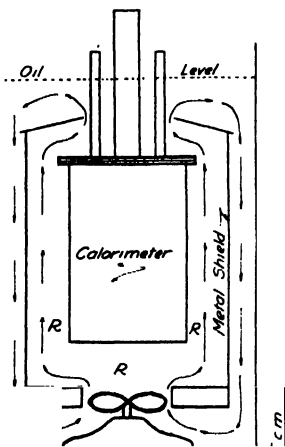


FIG. 3. Oil bath and calorimeter.

In some experiments to be described later, a bath was required which could be operated from temperatures below 0° to above 100° C. A design that proved very satisfactory is shown in Fig. 3. Kerosene was used for the lower temperatures and transformer oil for the higher. A small fan motor with blades cut short was mounted in the oil as shown and produced good circulation. The top of the bath was well covered with cotton wadding and the outside wrapped with asbestos paper. It was then packed in sawdust or a freezing mixture as required. The temperature was controlled by electric heating and by hot or cold water passed through a coil of copper tubing. All the heating or cooling took place in the space outside the metal shield and the oil was thoroughly mixed by the fan before reaching the calorimeter jacket. The space *R* was thus completely shielded from outside disturbances and the temperature gradient at the boundary was small.

Differential Thermocouples

Accurate temperature equality between the calorimeter and surrounding jacket was assured by means of a six-junction differential thermocouple of No. 34 copper and No. 32 constantan wire connected directly to a sensitive galvanometer which gave about 5 mm. deflection for a temperature difference of 0.001°C . In the case of the water calorimeter, the junctions were mounted in thin-walled glass tubes which were placed in the water of the calorimeter and of the bath.

In the earlier experiments with the copper calorimeter, one set of six junctions was distributed in two slots on opposite sides of the copper calorimeter and the other six junctions were placed three in each of two small oil-filled cavities (*D*, Fig. 2), on opposite sides of the jacket.

In both the above types of thermocouple, considerable uncertainty existed regarding the relative lag in temperature of the junctions behind that of the jacket and of the calorimeter, when the temperatures of the two were rising at the same rate. In order to eliminate this uncertainty, a completely symmetrical arrangement of the thermojunctions was used in the latter part of this work. Two three-junction couples were used in series and each of the four sets of three junctions was similarly mounted in a short piece of thin $\frac{1}{8}$ -in. brass tubing.*

Two vertical $\frac{1}{8}$ -in. holes about 2-in. deep were drilled close to the surface on opposite sides of each copper calorimeter and two pieces of $\frac{1}{8}$ -in. copper tubing were soldered to the jacket. The junctions fitted neatly in these holes and could be easily removed and replaced without damage. Each thermocouple was tested by placing its junctions in the two holes of one copper calorimeter and then heating it. Exact similarity of the junctions was indicated if the galvanometer gave no deflection.

Measurement of Bath Temperature

During the earlier part of this work the temperature of the bath was measured with a standard Beckmann thermometer with Reichsanstalt certificate. Some question having arisen about its reliability, a comparison was made between it and two other standard Beckmann thermometers (also with Reichsanstalt certificates) and also another standard thermometer with Bureau of Standards certificate. No two of these thermometers agreed in the measurement of a temperature difference. In fact, the two in closest agreement differed by about 0.015° in a 5° interval. The writers were not satisfied with this degree of accuracy and reluctantly decided to make their own temperature calibrations.

A platinum thermometer bridge of the Callendar-Barnes type (2) was built and calibrated. The bridge was so made that a one-degree change in a 20-ohm platinum thermometer corresponded to about 20 cm. of bridge wire.

**Johns-Manville Plastic Refractory Cement No. 20 proved to be satisfactory for holding and insulating the junctions in the tubes. It was suitable for all temperatures and when thoroughly dry the insulation between couple and case was over 10^8 ohms.*

The sensitivity of the galvanometer was such that there was no difficulty in making settings to 0.1 mm. or 0.0005° when the current through the thermometer was 0.005 amp.

The bridge wire of manganin was carefully drawn and tested for uniformity. No 4-cm. section was found to differ by as much as 0.1 mm. from any other 4-cm. section. The bridge coils of manganin had a negative temperature coefficient of about 0.000005 at room temperature and the larger ones were approximately compensated by copper wire in series. This compensation was quite unnecessary as far as this investigation was concerned for the coils were in an oil bath the temperature of which was kept sufficiently constant to avoid errors. Repeated calibrations of this bridge showed the coils to be constant to within 0.2 mm. of bridge wire.

Two platinum thermometers of about 20 ohms each were made. The first was from commercial platinum which proved to have a temperature coefficient of 0.00345 and $\delta = 1.59$. The second was of c.p. platinum having a temperature coefficient of 0.00391 and $\delta = 1.52$. Repeated determinations of the fundamental intervals of these thermometers indicated that they were reliable to within 0.01%.

These two thermometers were in good agreement in their measurements of temperature differences between 0° and 100° C. Both gave the same corrections for two of the Beckmann thermometers previously mentioned and showed that one read too high and the other too low by about 0.008° in 5° C. All the later measurements of the bath temperature were made with one or the other platinum thermometer and the writers have found no reason for questioning the reliability of these measurements. The earlier measurements made with the Beckmann were all corrected by the platinum thermometer calibration.

Furnace

A fairly satisfactory furnace for heating the specimen was made by using a 10-in. length of $\frac{3}{4}$ -in. heavy copper pipe with about 3-in. of glass tubing of the same internal diameter fitted to each end (Fig. 4). Wire and insulating material were wound on this and adjusted with extra turns of wire at the end until there was an 8-cm. space at the centre which did not show any temperature variation of over 0.2° at 300° C. when explored by a thermocouple, even when the specimen was not present. The resistance of the heating coil was about 130 ohms, and 0.6 amp. raised the temperature to about 325° C.

Cooler

In order to get specific heats below room temperature, a simple "cooler" was made by passing a $\frac{3}{4}$ -in.

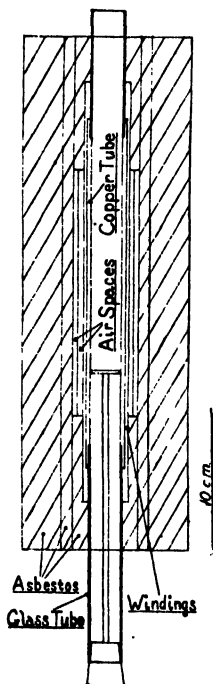


FIG. 4. Electric furnace for heating specimens.

brass tube through the centre of a can 6 in. in diameter and 12 in. long in which was placed ice or a freezing mixture. The specimen was suspended in the centre of the tube and could be dropped directly into the calorimeter in the same way as from the furnace.

Measurement of Furnace Temperature

The temperature of the metal specimen in the furnace or cooler was obtained by means of a single-junction thermocouple which was inserted in a hole drilled in the specimen. These holes extended practically the entire length of the copper and molybdenum specimens, but the writers were unable to drill more than 5 mm. into the tungsten even with a diamond drill. In some of the experiments at the higher temperatures the tungsten was surrounded by a copper shield which came well up on the thermocouple thus ensuring that it was at the temperature of the tungsten, but this produced no noticeable change in the results.

The e.m.f. of the thermocouple was measured in the usual way on a Leeds and Northrop type K potentiometer. The standard cell was kept in a thermostat and its e.m.f. was known by comparison with standard cells at McGill University.

Temperatures up to 350° C. could be satisfactorily measured with a couple of No. 34 copper and No. 32 carefully selected commercial constantan wire, and such couples were used in all the earlier experiments. The junctions were placed in thin-walled glass tubes, one of which was always in a large Dewar flask filled with ice. The couples were calibrated in steam and in melting tin, cadmium, and zinc. The metals were melted in cylinders of iron or carbon which just fitted into the furnace used for heating the specimens. Thus during calibration the couples were under exactly the same conditions as when used in measuring temperature. Another advantage of this arrangement was that the furnace gave such perfect control of the rate of cooling that at the melting point the temperature would not change by as much as 0.05° C. in 10 min.

The thermoelectric properties of pieces of commercial constantan wire even from the same spool differ considerably. Curve A, Fig. 5, is the calibration curve of one of these couples. The method suggested by Adams (1) has been used. The abscissas give the values of the e.m.f. in microvolts, and the ordinates the differences between these values and

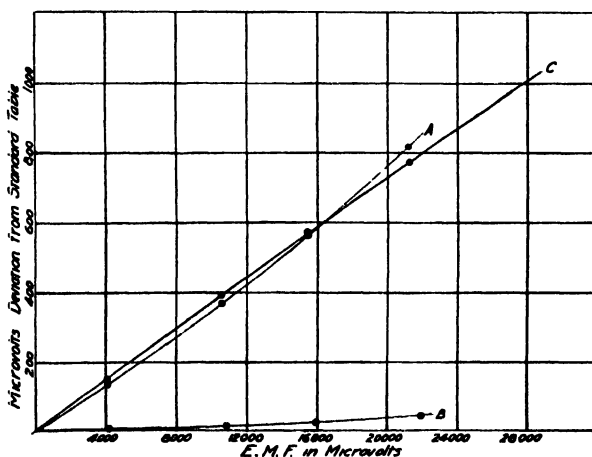


FIG. 5. Thermocouple calibration curves.

those given by Adams for the same temperatures. By the use of this deviation curve the temperature corresponding to any e.m.f. is readily obtained from the equation given by Adams or, more conveniently, from his very accurately calculated Calibration Table. Later it was possible to check these results with a couple made from special high grade constantan wire which was very kindly supplied by L. H. Adams of the Geophysical Laboratory at Washington. This wire was from the same lot as that used by him in determining his thermocouple calibration tables and equation (1). The close agreement of our calibration of this thermocouple with that given by Adams is shown in Table I and by curve *B*, Fig. 5, and confirms the

TABLE I

COMPARISON OF CALIBRATIONS OF THE HIGH GRADE CONSTANTAN COUPLE (FIG. 5, CURVE *B*)

Temperature, °C.	100	231.8	320.9	419.4
E.m.f. by Adams, microvolts	4276	11009	16083	22075
E.m.f. by authors	4274	10993	16055	22027
Difference	2	16	28	48

accuracy of our methods of calibration. The deviations of both thermocouples from the Standard Table are due to slight differences in the constantan alloy, but thermocouple *B* has two great advantages over *A*. In the first place, its deviation curve could be drawn to a larger scale, thus increasing the accuracy of the temperature determination, and in the second place, there was no danger of disturbing effects due to lack of homogeneity which are always possible when commercial constantan is used.

Since copper-constantan couples of fine wire deteriorate rapidly above 350° C., couples of platinum and platinum with 10% of rhodium and couples of alumel-chromel were tried. The latter were used in a large number of experiments, but all of these were finally rejected. Lack of homogeneity in the wires was apparently responsible for errors of as much as 1%.

It was finally decided to try at higher temperatures a couple of No. 30 copper and No. 28 constantan, also kindly supplied by Mr. Adams. This couple proved quite satisfactory up to 500° C. Its calibration curve taken after its use at this temperature differed by less than 0.1% from that taken before. Its deviation curve extrapolated to 500° C. is shown as *C*, Fig. 5. Adams' Calibration Table extending from 0° to 385° C. was calculated from the equation

$$E = 74.672t - 13892 (1 - e^{-0.00261t}).$$

In order to test the applicability of this equation up to 500° C., one junction of the couple and the platinum thermometer were placed close together in holes drilled in a piece of $\frac{3}{4}$ -in. copper rod. This was placed in the centre of the furnace and its temperature measured by both the thermocouple and the platinum thermometer at four points, the lowest being about the melting point of zinc at which temperature the e.m.f. of the couple was known. The result of this comparison is shown in Table II, and quite justifies the use of the couple for measurements up to 500° C.

TABLE II

	1	2	3	4
<i>T</i> , by platinum thermometer, °C.	418.8	445.5	471.1	501.3
<i>T</i> , by thermocouple, °C.	418.8	445.6	471.3	501.4

Specimens of Copper, Molybdenum, and Tungsten

All the specimens were in the form of cylinders from 1.5 to 1.7 cm. in diameter and from 2 to 4 cm. in length. Four specimens of masses about 34, 51, 69, and 89 gm. were made from commercially pure cold-rolled copper rod of density about 8.90 gm. per cc. The two specimens used at high temperatures were nickel-plated to prevent oxidization. The small amount of nickel deposited was known and corrected for when calculating the specific heat.

The writers had at their disposal only one cylinder of molybdenum and two of tungsten. All three were supplied through the kindness of the Research Laboratory of the General Electric Company and were supposed to be pure.

The specific heat of the molybdenum cylinder had previously been determined by Cooper and Langstroth (3). It had a mass of about 70 gm. and specific gravity of about 9.75. One cylinder of tungsten, of mass about 133 gm. and specific gravity 19.05, had been carefully swaged and polished. The other tungsten specimen, of mass about 83 gm. and specific gravity 17.3, was made from a block that had not been as carefully swaged and was not nearly as hard. In fact, a hole for the thermocouple was successfully drilled in it. The specific heat of this specimen seemed continually to increase when heated above 400° C. Finally it split open, revealing inside a considerable amount of greenish-yellow porous material, probably an oxide, which doubtless accounted for its low density and increasing specific heat. The results obtained with this specimen were therefore rejected.

Slight traces of oxidization were evident on the surfaces of the tungsten and molybdenum specimens, but not enough to cause a change in weight of 0.01 gm.

Thermal Capacity of Water Calorimeter

The water equivalent or thermal capacity of the water calorimeter was determined by two different methods. The first consisted merely in adding to the weight of water the water equivalent of the component parts of the calorimeter determined as follows:

	Mass, gm.	Sp. ht.	Water equiv., gm.
Copper calorimeter and stirrer	197.3	0.0915	18.05
Glass thermocouple tube	1.7	0.16	0.27
Thermocouple wire	1.0	0.097	0.10
Thermocouple cotton insulation	0.3	0.4	0.12
Paraffin wax	0.1	0.7	0.07
Air heated by calorimeter			0.06
Cooling due to evaporation			0.4
Total			19.1

In the second method the total thermal capacity in joules per °C. was determined electrically. About 60 ohms of cotton-covered manganin wire was wound on a thin copper cylinder and placed in the inside calorimeter vessel. Copper lead wires were brought out through holes in the cover and wound several times around the outside of the calorimeter to prevent any possible conduction of heat from the coil out to the jacket. Potential leads were soldered to these current leads just above the calorimeter and the four leads carried out through one of the tubes in the jacket cover. The current and potential were measured by standard methods, using a Leeds and Northrop type K potentiometer and standard cell. All resistances used were carefully calibrated. The current was adjusted to give a rise in temperature of about 0.25° per min. and the outside bath was heated electrically at the same rate, the differential thermocouple being kept balanced to 0.01°. The time, usually 15 or 20 min., was easily measured with a good watch to 0.2 or 0.3 sec.

The corresponding water equivalent was obtained by using the relations

$$1 \text{ calorie}_{15} = 4.1835 \text{ electrical joules}$$

$$1 \text{ calorie}_{20} = 4.180 \text{ electrical joules}$$

$$1 \text{ calorie}_{25} = 4.176 \text{ electrical joules}$$

From the result thus obtained it was necessary, in order to get the water equivalent of the calorimeter, to subtract the mass of water used and the water equivalent of the heating coil. The mass of water used was between 300 and 350 gm. The water equivalent of the heating coil was small and was obtained with sufficient accuracy as follows:

	Mass, gm.	Sp. ht.	Water equiv., gm.
Copper cylinder	15.37	0.0915	1.41
Manganin wire	2.54	0.097	0.25
Cotton insulation	0.97	0.4	0.89
Copper lead wire	0.37	0.09	0.03
Cotton insulation	0.07	0.4	0.03
Total			2.1

The mean of three such determinations of the water equivalent of the calorimeter was 19.3 gm. This is in better agreement with the previous value (19.1 gm.) than might be expected, the difference, 0.2 gm., being only 0.06% of the water equivalent of the calorimeter and contents. It is worth noting here that this close agreement is a further confirmation of the reliability of the writers' Beckmann calibration. It has already been pointed out in the section on differential thermocouples that balanced thermocouples do not necessarily indicate equality of temperature between the jacket and calorimeter when their temperatures are rising at the same rate. A careful investigation in the case of the water calorimeter indicated that this error might

possibly have caused an error of 0.5 gm. in the above water equivalent. If now the calorimeter is to be used for specific heat measurements, the water equivalent, determined electrically, has the very great advantage of including the above errors in such a way as to exclude them from the final result. In this case the calorimeter and Beckmann thermometer become merely transfer instruments.

Thermal Capacity of the Copper Calorimeters and the Specific Heat of Copper (Electrical Experiments)

The thermal capacity of each of the all-copper calorimeters was also determined by the above electrical method, except that both Beckmann and platinum thermometers were used to measure the bath temperature. No weighings were required and no corrections had to be made for air, thermocouples or Beckmann thermometer when the calorimeter was being used in specific heat determinations by the method of mixtures. In fact, the calorimeter, calibrated in this way without corrections, becomes an ideal transfer instrument.

Since these calorimeters are more than 99.5% pure copper, it is evident that we have here a method for determining the specific heat of copper for a small temperature change. This requires, however, the making of all those corrections which are unnecessary when the calorimeter is to be used merely as a transfer instrument. It was because this method of determining the actual specific heat of copper at a definite temperature seemed to be capable of high precision that the platinum thermometer and oil bath were constructed.

The accuracy of this specific heat determination depended upon the measurements of the electrical power supplied, the time, the rise in temperature, and the copper equivalent of the calorimeter which includes the thermocouples, heating coil, and surrounding air. The first three quantities were measured with an accuracy of 0.02% or better. The calculation of the copper equivalent of the No. 2 calorimeter is given below in order to show the size of the various quantities and the probable accuracy. The copper equivalent was obtained by multiplying the mass of each substance by the

	Weight, gm.	Copper equiv., gm.
Copper calorimeter	2953.8	2953.8
Brass hinges and screws	4.1	4.0
Brass tubing for thermocouple	4.6	4.5
Cement for thermocouple	0.12	0.3
Wire for thermocouple	0.4	0.4
Cotton insulation for thermocouple	0.12	0.5
Copper cylinder for heating coil	59.1	59.1
Manganin wire for heating coil	2.5	2.6
Copper lead wires for heating coil	0.3	0.3
Cotton insulation for heating coil	1.1	4.4
Air correction		0.7
Total		3030.6

ratio of its specific heat to that of copper. In spite of some uncertainty in the values of the specific heats of some of the materials, an error of as much as one gram in the total copper equivalent would seem improbable.

In all, over 200 determinations of the specific heat of these copper calorimeters were made at temperatures from -5° to 110° C. The experimental conditions were changed from time to time in order to discover and eliminate any constant errors. The temperature change for a single experiment was usually about four degrees when using a Beckmann thermometer, and eight or ten degrees when using the platinum thermometer. As previously mentioned, the oil bath made possible satisfactory adiabatic conditions at both the low and the high temperatures. The water bath was more convenient between 15° and 40° C.

Specific Heat by the Method of Mixtures ("Dropping Experiments")

Since the application of the adiabatic calorimeter to the determination of specific heat by the method of mixtures is well known, only a few experimental details will be given here.

At the beginning and end of each experiment with the water calorimeter the differential thermocouple was kept balanced for about 30 min. in order to measure the "creep" previously discussed, and a correction was applied in the usual way. When this correction was greater than about 1% the experiment was rejected. In general, no such correction was necessary for the all-copper calorimeters.

In order to make certain that there was thermal equilibrium between specimen and thermocouple, the furnace heating current was always adjusted until the temperature change was less than 0.1° in two minutes. The specimen was then transferred to the calorimeter by holding the furnace directly over the calorimeter and cutting one end of the fine wire supporting it, thus allowing it to fall freely.

Since the thermal capacities of all the calorimeters were determined electrically, the only corrections which had to be considered were those due to the loss of heat of the specimen during the time of fall, about 0.15 sec. Assuming the specimen to be at 400° C., a rough calculation indicated that the maximum loss due to radiation as from a black body would be not more than 0.1%, and that due to conduction not more than 0.05%. The actual loss was undoubtedly much less than this and was partially compensated by the hot air carried into the calorimeter with the specimen. These corrections were so small and uncertain that no attempt was made to apply them. However, an attempt was made to determine the magnitude of this loss experimentally by a difference method. One of the specimens was enclosed in a thin metal sheath and their combined thermal capacities determined and then that of the sheath alone, but the heat losses were too small to be determined in this way.

Experimental Results

Specific Heat of Copper between -5° and 110° C. by the Electrical Method

Table III gives the results (shown graphically in Fig. 7) obtained by the electrical method for the three copper calorimeters, together with some of the experimental conditions. Columns 1 and 2 give the temperature and experimental value of the specific heat in joules per gm. $^{\circ}$ C. In most cases these figures represent the mean of two or more determinations made at about the same temperature. Column 3 gives the value of the specific heat calculated from the empirical equations, 7J, 8J, 9J, given in the next section. Column 4 gives the difference between Columns 2 and 3. In Columns 5, 6, and 7 are indicated the bath, thermocouple, and thermometer used in the experiment, W, K, O standing for water, kerosene, and heavy oil baths respectively, 1 and 2 for the earlier and later forms of thermocouple, and B and P for Beckmann and platinum thermometers.

In the experiments in which thermocouple No. 1 was used, corrections of 0.0001 or 0.0002 have been applied when necessary to correct for errors due to "lag" of thermocouple. Other than this no corrections of any kind were applied to the simple, direct calculations.

TABLE III

SPECIFIC HEAT OF COPPER IN JOULES PER GRAM PER DEGREE C.

Large calorimeter

T $^{\circ}$ C.	Specific heat		Diff.	Bath	Couple	Thermometer	T $^{\circ}$ C.	Specific heat		Diff.	Bath	Couple	Thermometer
	Experimental	From equation 7J						Experimental	From equation 7J				
2.8	0.3797	0.3798	-1	W	1	B	44.5	0.3881	0.3882	-1	K	2	P
5.3	0.3805	0.3804	1	W	1	B	46.3	0.3884	0.3885	-1	W	2	P
7.8	0.3814	0.3809	5	W	1	B	47.0	0.3885	0.3886	-1	O	2	P
11.4	0.3821	0.3817	4	W	1	B	48.2	0.3891	0.3888	3	W	1	B
15.5	0.3828	0.3826	2	W	1	B	51.1	0.3895	0.3893	2	W	1	B
18.0	0.3831	0.3831	0	W	2	P	51.4	0.3893	0.3894	-1	O	1	P
18.2	0.3832	0.3832	0	W	1	B	53.9	0.3901	0.3898	3	W	1	B
22.1	0.3841	0.3839	2	W	1	B	54.9	0.3898	0.3900	-2	K	2	P
22.9	0.3841	0.3840	1	W	1	P	56.2	0.3899	0.3902	-3	O	2	P
26.3	0.3848	0.3848	0	W	1	B	57.5	0.3902	0.3904	-2	W	1	B
26.3	0.3848	0.3848	0	W	2	P	62.3	0.3911	0.3911	0	O	1	P
30.5	0.3856	0.3856	0	W	1	B	65.1	0.3913	0.3916	-3	O	2	P
33.3	0.3861	0.3862	-1	W	2	P	67.6	0.3919	0.3920	-1	K	2	P
33.8	0.3860	0.3863	-3	W	1	P	76.0	0.3931	0.3932	-1	O	2	P
34.0	0.3863	0.3863	0	K	2	P	77.9	0.3935	0.3935	0	K	2	P
34.9	0.3865	0.3865	0	W	2	P	87.0	0.3946	0.3948	-2	O	2	P
35.0	0.3870	0.3865	5	W	1	B	91.9	0.3954	0.3955	-1	O	2	P
38.6	0.3875	0.3871	4	W	1	B	96.4	0.3961	0.3961	0	O	2	P
41.9	0.3877	0.3877	0	W	2	P	99.8	0.3965	0.3965	0	O	2	P
44.1	0.3882	0.3881	1	W	1	P	106.6	0.3973	0.3973	0	O	2	P
44.2	0.3882	0.3881	1	W	1	B							

Medium calorimeter

T°C.	Specific heat		Diff.	Bath	Couple	Thermometer	T°C.	Specific heat		Diff.	Bath	Couple	Thermometer
	Experimental	From equation 8J						Experimental	From equation 8J				
-5.9	0.3779	0.3778	1	K	1	P	38.0	0.3871	0.3872	-1	W	1	B
-0.7	0.3789	0.3791	-2	K	1	P	39.2	0.3875	0.3874	1	W	2	P
1.1	0.3794	0.3795	-1	K	1	P	39.4	0.3875	0.3874	1	W	1	P
3.7	0.3802	0.3801	1	W	1	B	39.6	0.3876	0.3875	1	K	1	P
7.9	0.3812	0.3811	1	K	1	P	39.7	0.3876	0.3875	1	K	2	P
8.6	0.3813	0.3812	1	W	1	B	42.3	0.3880	0.3880	0	W	1	B
13.5	0.3823	0.3824	-1	W	1	B	45.1	0.3885	0.3885	0	W	1	B
15.5	0.3829	0.3828	1	K	1	P	45.1	0.3885	0.3885	0	K	2	P
17.3	0.3832	0.3832	0	W	1	P	45.4	0.3888	0.3885	3	W	1	P
17.9	0.3831	0.3833	-2	W	1	B	46.2	0.3885	0.3886	-1	O	2	P
20.4	0.3839	0.3838	1	W	1	B	46.5	0.3888	0.3887	1	W	2	P
21.3	0.3840	0.3840	0	W	2	P	48.3	0.3890	0.3890	0	K	2	P
21.7	0.3840	0.3841	-1	W	1	P	48.7	0.3891	0.3891	0	W	1	B
24.1	0.3848	0.3846	2	W	1	B	53.3	0.3900	0.3899	1	W	1	B
25.6	0.3849	0.3848	1	K	1	P	55.1	0.3901	0.3902	-1	K	2	P
25.7	0.3848	0.3848	0	W	1	P	58.1	0.3909	0.3907	2	O	2	P
26.4	0.3850	0.3850	0	W	2	P	65.1	0.3918	0.3918	0	K	2	P
28.3	0.3854	0.3854	0	K	2	P	68.4	0.3923	0.3923	0	O	2	P
28.6	0.3854	0.3854	0	W	1	P	74.4	0.3931	0.3932	-1	O	2	P
28.7	0.3855	0.3854	1	W	1	B	78.7	0.3938	0.3938	0	O	2	P
30.0	0.3857	0.3857	0	W	2	P	84.8	0.3946	0.3947	-1	O	2	P
32.1	0.3861	0.3862	-1	W	1	P	89.7	0.3954	0.3953	1	O	2	P
32.4	0.3863	0.3862	1	K	1	P	95.3	0.3960	0.3962	-2	O	2	P
33.3	0.3864	0.3864	0	W	1	B	99.8	0.3967	0.3967	0	O	2	P
33.6	0.3863	0.3864	-1	W	2	P	105.1	0.3973	0.3974	-1	O	2	P
36.5	0.3869	0.3869	0	K	2	P							

Small calorimeter

T°C.	Specific heat		Diff.	Bath	Couple	Thermometer	T°C.	Specific heat		Diff.	Bath	Couple	Thermometer
	Experimental	From equation 9J						Experimental	From equation 9J				
13.0	0.3817	0.3816	1	W	1	B	40.8	0.3870	0.3870	0	W	2	P
17.3	0.3825	0.3825	0	W	1	B	42.9	0.3875	0.3874	1	W	2	P
19.9	0.3831	0.3831	0	W	2	P	45.1	0.3878	0.3879	-1	K	2	P
21.7	0.3834	0.3834	0	W	1	B	47.9	0.3881	0.3883	-2	W	2	P
23.5	0.3838	0.3838	0	W	2	P	55.4	0.3896	0.3896	0	K	2	P
24.7	0.3841	0.3840	1	W	2	P	65.8	0.3913	0.3913	0	K	2	P
26.0	0.3845	0.3843	2	W	1	B	68.8	0.3915	0.3916	-1	O	2	P
29.4	0.3851	0.3850	1	W	2	P	75.8	0.3932	0.3927	5	O	2	P
30.5	0.3853	0.3852	1	W	1	B	79.0	0.3931	0.3932	-1	O	2	P
32.6	0.3856	0.3855	1	W	2	P	89.0	0.3945	0.3946	-1	O	2	P
34.6	0.3859	0.3859	0	K	2	P	98.1	0.3959	0.3958	1	O	2	P
38.1	0.3867	0.3866	1	W	2	P	106.0	0.3969	0.3968	1	O	2	P

Mean Specific Heat of Tungsten, Molybdenum, and Copper by the Method of Mixtures

Table IV contains a summary of the results obtained for the mean specific heats of tungsten, molybdenum, and copper between widely varying initial temperatures and a final temperature of 28° C. In the actual experiments

TABLE IV

MEAN SPECIFIC HEAT IN CAL₂₀ BETWEEN 28°C. AND INITIAL TEMPERATURE*Tungsten*

Initial temp., °C.	Calculated from equation 1C	With copper calorimeter	Diff.	With water calorimeter	Diff.	Initial temp., °C.	Calculated from equation 1C	With copper calorimeter	Diff.	With water calorimeter	Diff.
-19.7	.03120	.03124	4			185.6	.03230			.03222	- 8
0.2	.03136	.03128	- 8			200.3	.03235	.03240	5		
54.5	.03171	.03174	3			206.4	.03237			.03231	- 6
64.8	.03176	.03180	4			216.5	.03241			.03250	9
66.2	.03177			.03183	6	238.8	.03248	.03245	- 3		
66.3	.03177	.03167	-10			286.7	.03263			.03257	- 6
68.9	.03179			.03178	- 1	287.8	.03263			.03264	1
71.1	.03180	.03184	4			288.8	.03264	.03264	0		
75.6	.03182			.03171	-11	312.3	.03270	.03268	- 2		
76.3	.03182			.03165	-17	317.5	.03272	.03273	1		
92.1	.03190			.03181	- 9	336.4	.03277			.03267	-10
98.1	.03193	.03197	4			372.4	.03287	.03290	3		
110.4	.03199			.03206	7	417.2	.03299	.03300	1		
133.5	.03209			.03210	1	420.3	.03300	.03304	4		
140.6	.03212			.03201	-11	420.8	.03300	.03304	4		
142.7	.03213			.03203	-10	421.1	.03300	.03298	- 2		
145.9	.03214			.03225	11	422.0	.03301	.03305	4		
151.5	.03216	.03217	1			444.4	.03305	.03304	- 1		
164.9	.03221			.03216	- 5	469.0	.03312	.03309	- 3		
183.4	.03229			.03230	1	500.3	.03319	.03320	1		

Molybdenum

Initial temp., °C.	Calculated from equation 3C	With copper calorimeter	Diff.	With water calorimeter	Diff.	Initial temp., °C.	Calculated from equation 3C	With copper calorimeter	Diff.	With water calorimeter	Diff.
-19.6	.05928	.05925	- 3			226.4	.06310	.06327	17		
00.1	.05974	.05956	-18			234.1	.06317	.06323	6		
50.3	.06073	.06062	-11			235.3	.06319			.06290	-29
64.6	.06097	.06112	15			235.5	.06319	.06353	34		
66.4	.06101	.06111	10			241.1	.06324	.06326	2		
68.6	.06103			.06106	3	274.3	.06358			.06325	-33
75.8	.06115			.06133	18	275.7	.06360			.06329	-31
76.2	.06115	.06114	- 1			309.4	.06393	.06405	12		
83.5	.06128	.06113	-15			316.7	.06400			.06370	-30
91.0	.06138			.06132	- 6	320.3	.06403	.06405	2		
99.9	.06152			.06167	15	320.4	.06403	.06399	- 4		
104.0	.06158	.06156	- 2			372.9	.06452	.06450	- 2		
108.4	.06164			.06162	- 2	414.6	.06488	.06475	-13		
132.6	.06197			.06197	0	418.0	.06491	.06490	- 1		
143.0	.06210			.06167	-43	419.0	.06492	.06488	- 4		
146.3	.06215			.06196	-19	420.5	.06493	.06491	- 2		
153.9	.06225	.06235	10			424.3	.06496	.06501	5		
163.8	.06237			.06238	1	447.8	.06516	.06515	- 1		
190.0	.06268			.06261	- 7	470.3	.06534	.06530	- 4		
219.1	.06301			.06313	12	501.4	.06559	.06569	10		

Copper

Initial temp., °C.	Calculated from equation 5C	With copper calorimeter	Diff.	With water calorimeter	Diff.	Initial temp., °C.	Calculated from equation 5C	With copper calorimeter	Diff.	With water calorimeter	Diff.
-18.5	.09070	.09050	-20			200.0	.09491			.0950	1
0.1	.09122	.09114	-8			206.0	.09499			.0951	1
0.3	.09122	.09145	23			216.7	.09512			.0954	3
48.5	.09238	.09214	-24			234.9	.09535			.0954	1
52.2	.09245	.09243	-2			238.2	.09541	.09531	-10		
64.9	.09272	.09264	-8			238.6	.09541	.09541		.09528	-13
74.3	.09290			.0930	1	273.2	.09582			.0957	-1
74.8	.09291			.0930	1	275.0	.09584			.0958	0
75.3	.09293	.09303	10			286.5	.09598	.09599	1		
99.1	.09336	.09338	2			290.4	.09602			.0961	1
100.1	.09337			.0937	3	290.7	.09602			.0959	-1
108.3	.09351			.0936	1	310.8	.09626	.09613	-13		
130.4	.09388			.0940	1	314.8	.09630			.0963	0
144.0	.09409			.0942	1	323.1	.09639	.09622	-17		
151.0	.09420	.09416	-4			372.1	.09693	.09696	3		
151.2	.09420			.0943	1	414.0	.09735	.09722	-13		
162.7	.09437			.0943	-1	417.8	.09739	.09742	3		
174.5	.09454			.0951	6	417.9	.09739	.09728	-11		
179.3	.09461			.0950	4	446.4	.09766	.09762	-4		
185.8	.09470			.0949	2	469.9	.09789	.09796	7		
198.7	.09489	.09499	10			501.0	.09818	.09835	17		

the final temperature often differed from this by several degrees, but the temperature range was easily adjusted without appreciable error, since the specific heat is nearly a linear function of the temperature. The results obtained with the copper and water calorimeters are shown separately in Columns 3 and 5. In Columns 4 and 6 are given the differences between these and the values given in Column 2, which were calculated from the empirical equations, 1C, 3C, and 5C, given in the next section. No differences were detected in the values of the specific heat of the several copper cylinders used.

Equations for Specific Heat

Theory

The Debye (4) function for the specific heat at constant volume can be expanded in a series, the terms of which are multiples of even powers of θ/T , where T is the absolute temperature and θ the "characteristic temperature" of the metal. This expansion is valid for a large range of temperature, including that under investigation. The first two terms in this expansion are $A - C/T^2$. Thermodynamic theory gives for the difference between the specific heat at constant pressure and the specific heat at constant volume an expression approximately equal to a constant times T . It was therefore decided to see how closely equations of the form

$$C_p = A + BT - C/T^2$$

could be made to fit the experimental results.

This equation is directly applicable to the specific heat values given in Table III but not to those given in Table IV, since the latter are the mean values between 28° C. and various initial temperatures. The equation can, however, be readily transformed to apply to mean values as follows:

Let S_m be the mean specific heat between 301° K (28° C.) and T° K, and C_p be the true specific heat at T° K. Then

$$\begin{aligned} S_m(T - 301) &= \int_{301}^T C_p dt = \int_{301}^T (A + BT - C/T^2) dt \\ &= A(T - 301) + \frac{1}{2} B(T^2 - 301^2) - C \frac{(T - 301)}{301T} \end{aligned}$$

and so

$$\begin{aligned} S_m &= A + \frac{1}{2} B(T + 301) - C/301T \\ &= A + 155 \frac{1}{2} B + \frac{1}{2} BT - C/301T \\ &= A_0 + B_0 T - C_0/T. \end{aligned}$$

Equations from Data by Method of Mixtures

Large-scale *mean* specific heat curves were constructed from the data given in Table IV and the constants A_0 , B_0 , and C_0 determined by the "cut and try" method. The resulting S_m equations are given below. As indicated in Table IV the average deviation of an individual determination from the curve was about 0.1% when the copper calorimeter was used and about 0.2% in the case of the water calorimeter.

The constants A , B , and C of the C_p equations were then calculated using the above relations between the constants. The resulting equations are given below in 20-degree calories (C — equations) and in joules (J — equations) in order to facilitate comparison with the results of other investigators. Equations 2C, 4C, and 6C are also shown graphically in Fig. 6.

Tungsten

$$\begin{aligned} S_m &= 0.03248 + 0.00000164T - .43/T & (1C) \\ C_p &= 0.03199 + 0.00000328T - 129/T^2 & (2C) \\ C_p &= 0.1337 + 0.0000137T - 540/T^2 & (2J) \end{aligned}$$

Molybdenum

$$\begin{aligned} S_m &= 0.06250 + 0.0000060T - 1.20/T & (3C) \\ C_p &= 0.06069 + 0.0000120T - 361/T^2 & (4C) \\ C_p &= 0.2537 + 0.0000502T - 1510/T^2 & (4J) \end{aligned}$$

Copper

$$\begin{aligned} S_m &= 0.09486 + 0.0000068T - 1.50/T & (5C) \\ C_p &= 0.09280 + 0.0000136T - 452/T^2 & (6C) \\ C_p &= 0.3879 + 0.0000569T - 1890/T^2 & (6J) \end{aligned}$$

Equations for Copper from Data by Electrical Method

The curves 1, 2, and 3, Fig. 7, are plotted from the experimental values given in Table III for the large, medium, and small calorimeters respectively. The lower part of the curve for copper in Fig. 6 is added for the sake of comparison. The following three equations were obtained from large-scale curves by the "cut and try" method.

$$\text{Calorimeter 1} \quad C_p = 0.3889 + 0.0000569T - 1890/T^2 \quad (7J)$$

$$\text{Calorimeter 2} \quad C_p = 0.3891 + 0.0000569T - 1890/T^2 \quad (8J)$$

$$\text{Calorimeter 3} \quad C_p = 0.3884 + 0.0000569T - 1890/T^2 \quad (9J)$$

$$\text{Calorimeter 3} \quad C_p = 0.09292 + 0.0000136T - 452/T^2 \quad (9C)$$

The differences between the constant terms of equations 6J, 7J, 8J, 9J will be discussed in the next section.

Approximate Linear Equations

For some purposes a linear equation over a limited range of temperature is sufficiently accurate and much easier to evaluate than those above. The following linear equations are therefore given merely for practical convenience. They do not differ from the more exact equations 2C, 4C, and 9C by more than 0.2% over the indicated temperature range. C_p is in cal./gm.° C. and t is Centigrade temperature.

Temperature Range 0° to 100° C.

$$\text{Tungsten} \quad C_p = 0.03121 + 0.0000113t \quad (10C)$$

$$\text{Molybdenum} \quad C_p = 0.05926 + 0.0000344t \quad (11C)$$

$$\text{Copper} \quad C_p = 0.09076 + 0.0000415t \quad (12C)$$

Temperature Range 150° to 500° C.

$$\text{Tungsten} \quad C_p = 0.03200 + 0.0000047t \quad (13C)$$

$$\text{Molybdenum} \quad C_p = 0.06150 + 0.0000160t \quad (14C)$$

$$\text{Copper} \quad C_p = 0.09357 + 0.0000186t \quad (15C)$$

Results of Previous Investigators

It seems desirable for purposes of comparison to record here several of the more recently published equations for the specific heats of tungsten and molybdenum, and for the sake of completeness to add the two equations for copper given in the Introduction.

The following three equations for the specific heat of tungsten were published about the time, or soon after, this investigation was started:

$$C_p = 0.0319 + 0.000006t \text{ by Zwikker (13)}$$

$$C_p = 0.032036 + 0.0000045292t \text{ (Range } 100^\circ \text{ to } 900^\circ \text{ C.)}$$

$$\text{by Magnus and Holzmann (10).}$$

$$\text{and } C_p = 0.03199 + 4.848 \cdot 10^{-6}t - 11.74 \cdot 10^{-11}t^2 \text{ (Range } 0^\circ \text{ to } 1600^\circ \text{ C.)}$$

$$\text{by Jaeger and Rosenbohm (8).}$$

In 1927 Van Voorhis (12) published a paper on the work function of molybdenum in which he gives a summary of the values of its specific heat, and points out that the divergencies are so great as to render them useless. However, he decides upon the following linear equation as the most probable.

$$C_p = 0.0606 + 0.000028t.$$

More recently two other equations have been published,

$$C_p = 0.05973 + 0.00001619t \text{ (Range } 0^\circ \text{ to } 445^\circ \text{ C.) by Stern (11)}$$

$$\text{and } C_p = 0.0593 + 0.000013(t + 40) - 0.0265/(t + 40)^{1.66}$$

$$\text{by Cooper and Langstroth (3).}$$

The range given for the latter is -40° to 250° C. which is obviously impossible.

The following two equations for copper have already been given in the Introduction:

$$C_p = .0905 + .000048t \text{ (Range } 0^\circ \text{ to } 50^\circ \text{ C.) by Harper (5),}$$

$$\text{and } C_p = .0916 + .000025t \text{ (Range } 0^\circ \text{ to } 500^\circ \text{ C.) from I.C.T. "best" values.}$$

All the above equations show the wide divergencies, especially in the second term, which have already been discussed in the case of copper.

Discussion of Results

The three specific heat curves of Fig. 6 have the same general characteristics which have been briefly discussed in the Introduction. That these curves are far from linear and that the change in the slope with the temperature is much greater at low, than at high, temperatures is shown by the figures in Table V.

It is clear from these figures that neither a first nor a second degree equation will fit the experimental curves. It is not possible, therefore, to compare satisfactorily our results with those of others given in the previous section. It is perhaps enough to point out that the high but rapidly decreasing values of the slopes of the curves from 0° to 100° C. account in part for the variations in the second term of the

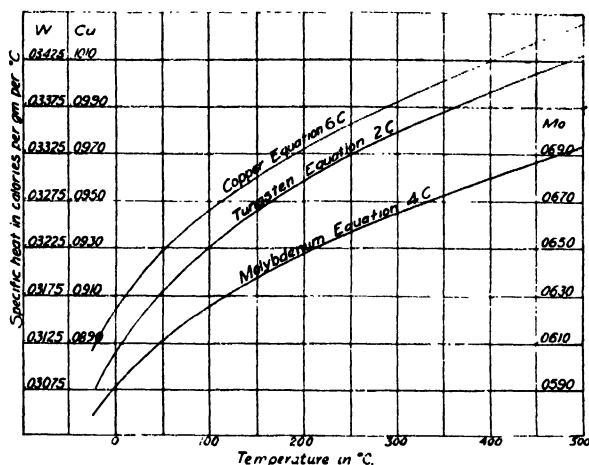


FIG. 6. Specific heats of tungsten, molybdenum and copper.

TABLE V
SLOPE OF CURVES

Temperature	0°	250°	500°
Tungsten	0.0000160	0.0000051	0.0000040
Molybdenum	0.000048	0.000017	0.000014
Copper	0.000058	0.000020	0.000016

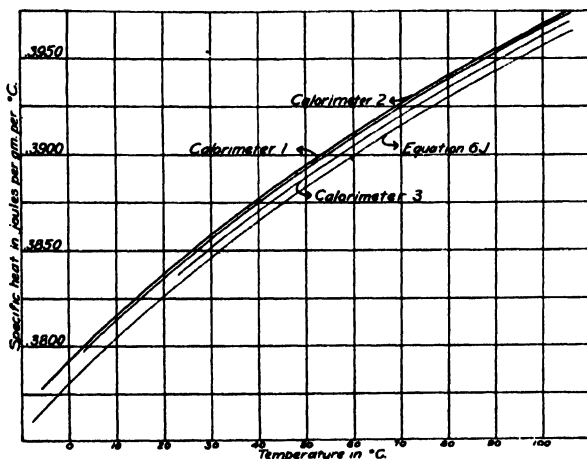


FIG. 7. Specific heats of copper calorimeters and cylinders.

equations obtained by different observers. It is also worth noting that these variations are not in general greater than those between our linear equations (10C, 11C, 12C) for the range 0° to 100° C. and the equations (13C, 14C, 15C) for the range 150° to 500° C.

It has been pointed out previously that the accuracy of the "dropping experiments" decreases rapidly as the initial temperature approaches the final temperature because of the smallness of the quantities to be measured. This means that the precision is small between 0° and 50° C.

where high precision is especially needed. On the other hand it is for this range of temperature that the "electrical method" is particularly suited, but unfortunately it could be applied only to copper. It is therefore of particular interest to compare the results for copper below 100° C. obtained by the two methods.

These results are shown graphically in Fig. 7 and by equations 6J, 7J, 8J, and 9J. The four curves were found to be parallel and therefore the *B* and *C* terms are the same for the four equations. The fact that no difference could be observed in the shape of the curves obtained by the two methods is a fine check on the reliability and accuracy of the results obtained not only for copper but also for tungsten and molybdenum where only one method was available.

It is next necessary to examine and, if possible, find an explanation for the variation in the constant terms of the four copper equations. It is seen from equations 6J, 7J, 8J, and 9J that the specific heats of the two cast-copper calorimeters are nearly the same and distinctly higher than that of the smallest calorimeter which was of cold-rolled copper of about 1% greater density. The consistency of the determinations under widely varying conditions, the fact that the specific heat curves of the three calorimeters are parallel and that all three were used in the determinations of the specific heat of the cylinders, all suggest that the differences in the specific heats are real and not introduced by experimental errors.

No attempt was made to obtain a chemical analysis of the copper, as it seemed very improbable that impurities were responsible for the differences in specific heat. Commercial copper wire and rod are usually over 99.9% pure and, according to the I.C.T., 1% of zinc changes the specific heat of copper only by about 0.01%. It was, however, suspected that the low density and high specific heat of the cast copper might be due to cavities in the castings containing more or less copper oxide. Support was given to this possibility by the finding of one rather large cavity during the machine work on the largest calorimeter. In order to settle this point, a tube containing about 100 millicuries of radium emanation was placed inside each calorimeter and the outside surface covered with photographic film. An exposure of from 15 to 25 min. produced a picture showing clearly every detail of the calorimeter, but there were no signs of any cavities or irregularities in density.

Theory shows that the specific heat is a function of the density and that it would be possible to calculate the change in specific heat corresponding to a 1% change in density if the other physical properties of copper were sufficiently well known. The required constants are too uncertain to give any real significance to such a calculation, but the available data point to a greater difference in the specific heat than that actually observed.

The specific heat of the small copper cylinders is seen to be about 0.15% less than that of the smallest calorimeter, but in this case there is practically no difference in density and both are made from cold-rolled copper rods. It

is natural in this case to suspect that the difference is due to the different experimental methods used, since the electrical method was used for the copper calorimeter and the method of mixtures in the case of the small cylinders. As already mentioned, no correction has been made for the small and uncertain loss of heat from the cylinder during its time of fall. Rough calculations indicate that this loss should be well below 0.1% for temperatures under 100° C. It does not seem, therefore, that this loss can account for the entire difference of 0.15%. It is, of course, possible that some other error of the order of 0.1% has crept into one or the other method of measurement; or variations in some of the physical properties of the two samples of copper may be responsible for the difference.

Equation 9C (the specific heat equation for the small copper calorimeter) is selected for the Abstract and as a basis for the approximate linear equations, because the copper of which it is made is more nearly a standard commercial product than copper castings and because it is believed that the "electrical experiments" are more accurate than the "dropping experiments."

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FOWL PARALYSIS (NEUROLYMPHOMATOSIS GALLINARUM) IN CHICKS UNDER THREE MONTHS OF AGE¹

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Abstract

Fowl paralysis is generally considered to be a disease of birds over three months of age. In the present paper data are presented on 45 out of 244 chicks (18.4%), which developed clinical symptoms of fowl paralysis before 90 days of age. Of these, 20 were inoculated and 25 were not inoculated. Forty-two of the 45 chicks (93.3%) showed lesions in the nervous system. Nine chicks (20%) showed lymphomatous tumors.

Seventeen of the 45 cases (37.7%) occurred in chicks less than 60 days of age; the same number of cases occurred between 60 and 74 days of age; and 11 cases (24.4%) occurred between 75 and 89 days of age. The mean age of all chicks that developed paralysis was 64.4 days.

The fact that typical fowl paralysis occurred in one chick at 37 days, and in several from 40 to 44 days of age, would indicate that the disease may develop at a very rapid rate.

Fowl paralysis (*Neurolymphomatosis gallinarum*), although known to affect adult birds, is generally considered to be a disease of young birds. Thus it has been noted that spontaneous cases of clinical fowl paralysis may occur in fowls 3 to 18 months of age. The disease, however, appears to be prevalent in pullets which are approaching maturity or have just started to lay, the largest percentage of cases usually occurring between three and eight months of age.

Although the precise "incubation period" of fowl paralysis is not known, it is thought to be about three months. This figure is based on the evidence that spontaneous or experimental cases of fowl paralysis usually do not appear until the birds are about three months old.

Review of Literature

Pappenheimer, Dunn and Cone (11) record in detail the age incidence of fowl paralysis of the Leghorn flock at the Storrs Experimental Station during the years 1922, 1924 and 1925. In 1922 and 1924 the disease appeared after the

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birds were three months old, and in 1925 after they were four months of age. The disease continued to appear until the birds were 16 months old. The highest mortality was regularly encountered in the first five months after the appearance of the disease, or roughly, between the months of August and December. At the Storrs' egg laying contest, out of 3800 pullets which came under observation when about seven months old, 97 developed paralysis between 7 and 18 months of age. In partial histories obtained from several farm flocks it was determined that fowl paralysis might make its first appearance as early as ten weeks or as late as the fifth or sixth month of age.

Considerable variation in the age incidence of fowl paralysis has been also noted in the successful transmission experiments reported by Pappenheimer *et al.* Thus chicks, two to five days old, inoculated with the same material upon the same day, developed symptoms of fowl paralysis at different ages. The earliest clinical case was observed at 10 weeks of age, while the majority of the birds developed fowl paralysis between 110 and 205 days of age.

Kaupp (7) considers that birds from 4 to 12 months of age are most susceptible to fowl paralysis. Stafseth and Johnson (12) state that in Michigan fowl paralysis is the most serious ailment affecting birds from 4 to 14 months. Doyle (4) states that the first symptoms of paralysis usually appear when the birds are from three to six months of age, but birds under three, and more than eight, months of age may become affected with the disease. He makes the important observation that "the peculiar type of blindness, which has such a close flock association with paralysis, usually does not occur in birds much less than 8 months of age". The earliest age of occurrence of paralysis in the literature is recorded by Doyle, who reports that a cockerel showed clinical symptoms of fowl paralysis at 47 days of age. Thomas (13) reports that an outbreak of fowl paralysis started in a flock of chickens about two to four months old and reappeared when the birds were 10 to 12 months old. In another flock the disease appeared between the ages of three and five months, while in a third flock fowl paralysis appeared in pullets three to six months old. Emoto and Miyamoto (5) state that in Japan the earliest spontaneous case was observed at 4 months, and that birds about 12 months of age are the most frequently affected. Newsom (10) considers fowl paralysis to be a disease of "mature fowls, from 5 to 15 months of age". McGaughey (9) states that fowl paralysis usually first appears in young pullets from four to eight months. Beach (1) notes that fowl paralysis occurs in fowls between the ages of four and eight months, although it has been observed in pullets three months old and in hens up to 18 months of age. Beaudette and Hudson (2) report that fowl paralysis occurs most often between the third and eighth months of age. They introduce a table showing that during 1929-1930 and 1930-1931 cases of fowl paralysis were submitted every month of the year for diagnosis at the New Jersey Agricultural Experiment Station. Warrack and Dalling (14) state that fowl paralysis is found to occur mostly in young birds, from 8 or 9 weeks up to 18 months of age, but that "the most commonly affected birds are those from 3 to 5 months old". Johnson (6)

reports that fowl paralysis occurs most frequently in "young birds between 3 and 18 months of age". A few cases outside of these limits have been noticed. Mayhew (8) reports cases of paralysis in chicks as young as eight weeks, and as old as eight months of age. Twelve out of 27 cases developed before three months of age.

The purpose of this paper is to record observations on the incidence of fowl paralysis in chicks under three months of age.

Material and Methods

The chicks used for this study were hatched in several lots from eggs obtained from a Rhode Island Red and Black Orpington flock of birds in which heavy losses from fowl paralysis had previously occurred. When the chicks were 24 hours old they were leg-banded and transferred to battery brooders, where they were kept from eight to ten weeks. The chicks were then confined in small pens with wire floors, or pens with wooden floors with peat-moss litter. Throughout the experiment water and a balanced mash were kept before the chicks at all times.

The house in which the chicks were confined was isolated from other buildings and, with the exception of the attendant, was not visited by anyone. At the time the first lot of chicks was put in the battery brooders there were no paralyzed birds in the house. The premises were cleaned and disinfected before the experiment was started.

About one-half of the chicks (1-7 days of age) were inoculated by various routes with suspensions of tissue from the nervous system of birds affected with fowl paralysis. All chicks showing clinical symptoms of the disease were kept under observation for several days before they were killed and subjected to a careful post-mortem examination. With a few exceptions, gross lesions characteristic of fowl paralysis were found in the nervous system of affected birds. A number of birds also showed lymphomatous tumors. As, in the writers' experience, the typical lesions found in clinical cases of fowl paralysis always yielded the same histopathological picture, it was not deemed necessary to make a microscopic examination of every affected chick. However, histopathological studies were made of several birds to confirm microscopically the diagnoses made on the basis of clinical symptoms and lesions found on post-mortem examination.

Data

Out of 244 chicks forming the 10 lots under observation, 22 were killed or died from causes other than fowl paralysis. Their ages varied from 47 to 86 days, the majority being about 75 days old. None of these chicks showed lesions characteristic of fowl paralysis. On the other hand, 45 chicks (18.4%) developed clinical symptoms of the disease before they reached 90 days of age. Of these, 20 chicks were inoculated and 25 were not inoculated.

With the exception of four chicks which developed paralysis of the wing (Table I, Chicks Nos. 691, 324, 301 and 664), all the chicks showed typical paralysis of one or both legs. Two of the 45 chicks died and the rest were killed.

The results of the post-mortem examinations are presented in Table I, which shows that gross lesions were found in the nervous system of all but three chicks. Moderate to extreme changes were noted in the posterior root ganglia of the spinal cord in the thoracic region, in the corresponding segments of the spinal cord, in the brachial plexus, in the lumbosacral plexus,

TABLE I
DISTRIBUTION AND EXTENT OF GROSS LESIONS IN 45 CHICKS SHOWING CLINICAL SYMPTOMS OF FOWL PARALYSIS

Number of chick	Age in days when symptoms of paralysis were noticed	Lesions in lumbosacral plexus and sciatic nerves	Lesions in brachial plexus and posterior root ganglia in thoracic region	Lymphomatous tumors	Number of chick	Age in days when symptoms of paralysis were noticed	Lesions in lumbosacral plexus and sciatic nerves	Lesions in brachial plexus and posterior root ganglia in thoracic region	Lymphomatous tumors
675	37	++	+++	X	NN	66	++		
508	40		+	X	784	66	?	+	
517	42	++	++	X	977	69		+++	
704	44	+	++		2227	70	+	+	
677	44	+	++		324	70	++	+++	
649	47	+	+++	X	301	70	++	+++	
671	47	+++	+++		605	71	++	++	
308	50	+	++		524	71	+	+++	
508	52		+++		349	72	?	++	
781	52		+		450	72	++	?	
650	52	++	+		144	72	++	+	X
2225	55	+++	+++		664	73	+	++	
347	55	++	+++		772	76	++		
521	55		++		657	77	++	++	
778	57	++	++		879	77			
785	57	+	+	X	496	78	++	++	
304	58	++	+++		3702	78	++	+	
691	60		++		328	78		++	
864	62	++	+		518	80		++	
516	63		+		2397	81	+++	++	
865	65			X	48	82	++	++	X
769	65	+	+		869	84	?	?	X
					506	89	++	?	

NOTE:—?, lesions doubtful; +, moderately thickened and translucent; ++, considerably thickened and translucent; +++, enormously thickened and translucent; X, lymphomatous tumors present.

and in the sciatic nerves.* The distribution of the lesions according to size is shown in Table I. It will be seen that there was a preponderance of +++ and ++ lesions. Considering the fact that the chicks on the average were under 65 days of age, it is remarkable that such large lesions could develop in a comparatively short period of time without markedly influencing the rate of growth or the health of the chicks before the actual onset of paralysis.

*The gross lesions found in the brachial plexus and posterior root ganglia of the spinal cord in the thoracic region, and in the lumbosacral plexus and sciatic nerves, will be reported in the text as being found in the "brachial region" and the "lumbosacral region" respectively.

From an examination of Table I it will be seen that gross lesions were found more often in the brachial region than in the lumbosacral region. This bears out the writers' general observation that gross lesions are more commonly found in the brachial region than in the lumbosacral region, in spite of the fact that clinical paralysis of the wings is much rarer than paralysis of the legs. Thirty-nine chicks (86.6%) showed gross lesions in the brachial region as compared with 31 chicks (69.1%) showing lesions in the lumbosacral region. Altogether, 42 of the 45 chicks (93.3%) showed lesions in either the brachial or lumbosacral regions or both. It should be further noted that 9 (20%) of the chicks showed lymphomatous tumors.

TABLE II
THE DISTRIBUTION OF 45 CASES OF FOWL PARALYSIS ACCORDING TO AGE

Age in days	Inoculated chicks		Non-inoculated chicks		Total	
	Number	%	Number	%	Number	%
35-39	—	—	1	4.0	1	2.2
40-44	2	10.0	2	8.0	4	8.9
45-49	2	10.0	—	—	2	4.4
50-54	1	5.0	3	12.0	4	8.9
55-59	3	15.0	3	12.0	6	13.3
60-64	1	5.0	2	8.0	3	6.7
65-69	3	15.0	2	8.0	5	11.1
70-74	3	15.0	6	24.0	9	20.0
75-79	3	15.0	3	12.0	6	13.4
80-84	1	5.0	2	8.0	3	6.7
85-89	1	5.0	1	4.0	2	4.4
Total	20	100.0	25	100.0	45	100.0
Mean	64.0 days		64.8 days		64.4 days	

Table II shows the actual and the percentage distribution of the 45 cases of fowl paralysis according to age. It will be seen that the earliest case occurred between 35 and 39 days of age, or to be exact, at 37 days (Fig. 1). Seventeen of the 45 cases (37.7%) occurred in chicks less than 60 days of age, the same number of cases occurred between 60 and 74 days of age, and 11 cases (24.4%) occurred between 75 and 89 days of age.

On the basis of the total number of chicks under observation (244), about 7% developed paralysis before 60 days of age; 7% between 60-75 days of age; and 4.5% between 75-90 days of age. The mean ages of the inoculated and non-inoculated chicks were 64.0 and 64.8 days respectively. The mean age of all the chicks which developed paralysis was 64.4 days.*



FIG. 1. Chick No. 675 at 37 days of age, showing clinical symptoms of fowl paralysis.

Two of the 9 cases which showed lymphomatous tumors occurred in chicks under 2 months of age; in fact, one as early as 37 days of age. This is in agreement with the writers' previous observation (3) that the "incubation periods" of fowl paralysis and lymphomatous tumors are similar.

Discussion

The data show the very early age at which clinical cases of fowl paralysis may appear. As far as the authors are aware, this is the first time that a case of clinical paralysis occurring as early as 37 days of age is recorded. Although several investigators mention the fact that fowl paralysis may occur in chicks at two months of age, the condition is considered as exceptional. As already pointed out in the review of the literature, fowl paralysis is regarded as a disease affecting primarily chicks over three months of age. The data presented in this paper on the contrary show that a comparatively large number of cases of fowl paralysis may occur in chicks under three months of age.

In connection with the age incidence of fowl paralysis in the 45 chicks, there appears to be no consistency in the distribution (Table II). The means, standard deviations and coefficients of variation of the inoculated and non-inoculated chicks are as follows.

	Inoculated	Non-inoculated
Mean age	64.000 \pm 1.986	64.800 \pm 1.517
Standard deviation	13.155 \pm 1.408	13.200 \pm 1.254
Coefficient of variation	20.525 \pm 2.286%	20.370 \pm 2.012%

It would thus appear that inoculation of chicks at 1-7 days of age had no effect either on the mean age of the appearance of fowl paralysis or on the age distribution of the 45 cases.

The data in respect to age variation agree with those of Pappenheimer, Dunn and Cone (11) and of Warrack and Dalling (15), who report considerable variability in the time interval between inoculation and the appearance of clinical symptoms of fowl paralysis.

From an examination of Table I it will be seen that there does not appear to be any correlation between the "incubation period" of the disease and the extent of the gross lesions. Thus chick No. 675 showed correspondingly just as large lesions at 37 days as chick No. 2397 at 81 days. Furthermore, from the clinical observations it is evident that the degree of paralysis is not commensurate with the extent of the gross lesions as revealed by post-mortem examination. It is obvious, therefore, that symptoms of paralysis are only incidental and an external manifestation of an insidious disease. The fact that typical fowl paralysis with gross lesions may develop as early as 37 days of age suggests that the disease may attack chicks at a very early age and that the disease may develop at a very rapid rate.

Acknowledgment

The authors wish to express their great indebtedness to the following feed organizations for their generous contributions of feed and other supplies used in this and other studies of fowl paralysis at the University of British Columbia; The Brackman-Ker Milling Company, Limited; Buckerfield's, Limited; McLellan and McCarter, Limited; and The Vancouver Milling and Grain Company, Limited.

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SWINE ERYSIPELAS¹

By J. S. FULTON²

Abstract

Swine erysipelas has been recognized in widely separated districts in Saskatchewan, the disease having appeared in the acute, sub-acute and chronic forms.

The organism has been isolated from a number of cases and positive serological reactions obtained in others.

The organism isolated has been used to inoculate healthy pigs and in this way the disease has been reproduced.

As a result of the evident high mortality in the swine herds of Saskatchewan, it was deemed advisable to make a systematic study of the diseases common to the province that we might determine the nature of the affections causing the heavy losses.

During the years this work has been in progress it has become ever more apparent that the number of deaths gives a very inadequate picture of the total losses which are principally due to the large percentage of unthrifty animals usually designated as "runts". Investigation has revealed the fact that many of our so-called runts suffer from a specific disease known as swine erysipelas, in which the correct diagnosis has not been made because of the lack, or variety, of symptoms presented by the infected animals.

History

Swine erysipelas was first studied by Pasteur and Thuillier in 1882, and although these investigators did not establish the etiology of the disease, they did contribute much to the information available at that time.

The causative organism of swine erysipelas was isolated by Löffler in 1885.

Occurrence

Swine erysipelas occurs in all European countries, certain territories being heavily infested, so that outbreaks occur with regularity each season. The disease is most prevalent during the summer months, it abates towards fall, while only sporadic cases are observed during the winter.

Cause

The causative organism of swine erysipelas, *Erysipelothrix rhusiopathiae*, is a short, very slender, straight or slightly curved bacillus. In suitable liquid media, such as serum broth, the organism appears in long branching forms; to the naked eye, growth in this medium appears at first slightly cloudy, later granular and, as coalescence of the individual granules occurs, the growth falls to the bottom leaving the supernatant liquid clear.

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Types

Swine erysipelas presents a great variety of clinical types usually classified as acute, chronic and cutaneous. All of these have been observed in Saskatchewan, together with a sub-acute type presenting symptoms which in severity are midway between those of the acute and chronic. The chronic form is the most prevalent in Saskatchewan.

The Acute Form

In this type death generally occurs in a few days and the symptoms—fever, vomiting and lassitude, are sufficiently definite to permit a clinical diagnosis to be made.

The Chronic Form

Here diagnosis is difficult, either because of the lack of definite symptoms, or because of the great variety. It is so insidious in onset that it may be present in a herd for some time before any suspicion arises that there is anything wrong. The litter develops well until the pigs are two or three months old, when they cease to put on weight in spite of good food and care. The skin loses that bright, clean appearance indicative of good health and assumes a dirty, dry condition, while the hair becomes long and shaggy. We have found that pigs about six months old, suffering from the chronic form of swine erysipelas, often weigh no more than 35 to 50 lb., while healthy animals on the same farm weigh 200 lb. at the same age. Too often in the past these cases have been considered due to parasitism and the true cause has never been determined. In many of the chronic cases no more definite symptoms than those described above are presented and are so indefinite that diagnosis is impossible without laboratory tests, consequently such cases are unsuspected and become a prolonged source of infection.

Together with this unthrifty appearance some infected individuals show a dry, crusty eczema over the back, on the ears and sometimes around the lower joints of the limbs.

Erysipelothrix rhusiopathiae has evidently a decided affinity for joints, as in sub-acute or chronic swine erysipelas the infection often becomes localized in the joints of the limbs, setting up an acute or chronic arthritis. No difficulty has been experienced by the writer in isolating the causative organism in pure culture from such joints.

In cases where arthritis is acute there is a marked stiffness, and if forced to exercise, the animals show evidence of great pain. Should the joints of both hind limbs become involved the animals lose control of the hind quarters so that the condition is often described as paralysis, the true cause never being determined. It is usually the stifle, or hip joint, or both which are affected when complete loss of control of the hind limbs occurs.

In these cases the synovial sac is much enlarged and distended with fluid, which is turbid in appearance. The membrane is greatly thickened and

villus processes hang from the surface. The inner lining also shows numerous outgrowths, parts of which become detached and may be found floating in the fluid (joint mice).

The tissue surrounding the joint is usually edematous and this condition may extend a considerable distance from the focus of infection. In one case where the hip joint was involved the edema was readily discernible from the joint down to a point halfway between the stifle and the hock. This type of arthritis is usually confined to swine suffering from sub-acute erysipelas and although most animals ultimately succumb they may live for a considerable time.

Chronic arthritis may follow the acute but is often chronic from the beginning. Both in experimentally produced and in field cases of chronic swine erysipelas primary chronic arthritis has been observed. In these cases little pain or stiffness is evidenced until the process has developed to such an extent that the entire bony structure of the joint is changed and its free movement greatly impaired.

The bones of the carpal and tarsal articulations are more often affected than those forming the other joints of the body. The lesions observed have varied from a slight roughening of the free surface of one or two of the bones to marked outgrowths affecting all the bones comprising the joints.

In the more advanced cases, even although ankylosis does not occur, the joint may be rendered quite immobile through the interlocking of the bony outgrowths from the various bones.

The membrane of the synovial sac and the tissue surrounding the joint may be somewhat thickened but there are no marked changes such as are apparent in the acute type of arthritis already described. The synovial fluid may be more viscid than that found in normal joints but it is usually quite clear in appearance.

Endocarditis

Hutyra and Marek (1) state that chronic erysipelas "occurs most frequently as chronic erysipelatos-endocarditis in animals which have recovered from the acute affection. After the disappearance of the acute manifestations the pigs are usually lively and have a good appetite for a time, but on careful observation it may be noticed by comparison that they are stunted in their development."

In Saskatchewan chronic erysipelas has appeared in herds where the acute form never did occur and it has been reproduced experimentally by inoculating healthy pigs with cultures of *Erysipelothrix rhusiopathiae* secured from the joints of chronic field cases. The experimental animals so treated did not show any suggestion of acute erysipelas, but several weeks after inoculation they began to lose weight, assume an unthrifty appearance and later developed joint lesions from which a pure culture of the causative organism was recovered.

Endocarditis, which has been described as a characteristic lesion in chronic swine erysipelas, is not at all constant in the primary chronic cases we have examined. The valves in some instances have been slightly thickened, but vegetations have not been observed. Cultures made from the valves which appeared to be abnormal have yielded negative results.

Other Effects

The most constant change observed from gross examination of the organs of animals affected with chronic swine erysipelas is the enlargement of the spleen. This organ is usually dark in color and its surface is studded with elevations varying from the size of a pea to that of a large bean.

Microscopically the spleen shows marked congestion. The blood vessels are greatly distended and the spaces within the pulp are filled with red and white blood cells; the Malpighian bodies become indistinct.

The glomeruli of the kidney show marked congestion, the epithelium of the tubules is disintegrated and in some areas there is an excess of connective tissue.

Foci of granular degeneration appear throughout the liver tissue, interspersed with areas which are fairly normal.

Differential Diagnosis

The possibility of confusing parasitism and swine erysipelas must always be borne in mind especially in these cases of chronic erysipelas where no definite symptoms are presented.

In cases where chronic arthritis develops, the lower joints of the limbs may become so enlarged as to give the impression that the long bones are curved and that the animal is affected with rickets.

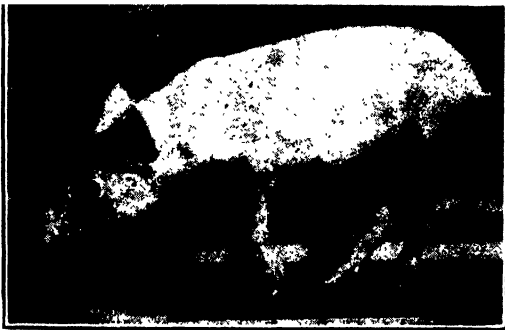


FIG. 1.

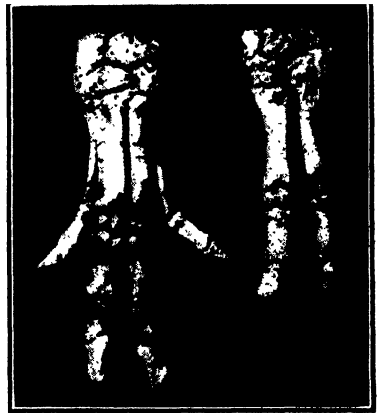


FIG. 2.

FIG. 1. Pure-bred Yorkshire, three months old, suffering from chronic swine erysipelas. Weight, 17½ lb. *Erysipelothrix rhusiopathiae* isolated from synovial fluid of the left carpal articulation. FIG. 2. Osteo-arthritis of carpal joints of pigs affected with chronic erysipelas. (Photographs by R. K. Baker).

The dry, crusty eczema, so common in animals infected with *Erysipelothrix rhusiopathiae*, may be quite readily confused with mange or sun scald. During the course of this investigation we have not attempted making a diagnosis of swine erysipelas without either isolating the causative organism or making use of serological tests on animals which could not be destroyed.

Tests of the Organism

The writer wishes to acknowledge a very complete report on two cultures submitted to Dr. J. B. Buxton, Institute of Animal Pathology, Cambridge. These cultures, secured from the joints of pigs suspected of having chronic swine erysipelas, have been compared with an authentic strain of *Erysipelothrix rhusiopathiae*, and apart from minor differences, have been found to be indistinguishable.

The strain described as "Runc", with which they were compared, was isolated from a pig suffering from the acute form of swine erysipelas and was pathogenic for mice in a dose of 0.00001 cc., while 24-hr. broth cultures from the original seed material of the Saskatchewan strains were lethal in doses of 0.1 cc., but not with smaller amounts. After passage through pigeons, however, the Saskatchewan strains, known as "Latta" and "Graham", became much more virulent, in fact quite comparable to the "Runc" strain. The same quantity (0.00001 cc.) of the 24-hr. broth culture, inoculated intraperitoneally, killed mice in four and six days respectively.

Dr. Buxton also carried out serum protection experiments using a fixed dose of anti-erysipelas serum with varying amounts of virulent cultures. This serum gave protection to mice receiving 10,000 minimum lethal doses of the "Latta" culture, 1000 of the "Graham" and 1000 of the "Runc" strains. Controls, receiving the same quantity of normal horse serum as was given of the anti-erysipelas serum, did not survive. Other controls receiving no serum also died. These results were confirmed by the application of serological tests *in vitro*.

Acknowledgment

The author wishes to acknowledge personal communications from L. T. Giltner, Pathological Division, Bureau of Animal Industry, Washington, D.C. which proved of great assistance in carrying out the work in the early stages of the investigation.

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THE PARASITIC HELMINTHS OF CANADIAN ANIMALS

I. THE CESTODARIA AND CESTODA¹

BY ROBERT ARNOLD WARDLE²

Abstract

One adult Cestodarian, 53 adult Cestoda and 24 larval Cestoda are known to occur in Canadian animals. The majority of the Cestoda are distributed among the Dibothriocephaloidea and Taenioidea.

Of the Cestoda, 29 of the adult species are represented in Europe, 14 are recorded elsewhere only in the United States, 6 are recorded only in Canada and 4 are unassignable. Of the 24 larval forms, 14 and one doubtful form are also European, 3 and two doubtful forms are recorded elsewhere only in the United States, 2 occur also in Japanese waters and 2 are not assignable.

An attempt is made to evaluate the economic status of the cestodes of the area.

Introduction

The scantiness of published information concerning the internal parasites of Canadian animals has hitherto precluded the preparation of a comprehensive report upon the incidence of endoparasitic helminths in the area. A considerable range of cestodarian and cestodan forms has now been recorded, however, and this information, supplemented by an accumulation of observations made by the writer and his coworkers upon the cestodes and nematodes of fishes, birds and mammals, appears sufficient to justify the presentation of the following survey. No claim towards completeness can be made. The cestodes of the domesticated mammals and birds and of the wild game animals are scarcely known. Very little information is available concerning the nematode parasites of any host group, and no information at all is available concerning the Acanthocephala.

The report may be of value, however, in directing the attention of other workers in parasitology to those fields in which further information is desirable, and in stimulating further investigations in this important branch of applied zoology.

The classification of the Cestodaria and Cestoda adopted here is, in broad outline, that proposed by Southwell (53) but the two groups will be regarded as distinct classes of the Platyelmia and no attempt will be made to subdivide them into orders. The Cestodaria comprise two families, the Cestoda comprise 30 families. These families are all familiar to the parasitologist, and diagnoses of them and of their component genera have been published by Meggitt (36) so that they need not be defined here. In order to keep the list of literary references within bounds only the more recent and comprehensive references that concern each superfamily will be alluded to. The references given under the heading of each species will be the Canadian references only.

¹ Manuscript received January 18, 1933.

Contribution from the Department of Zoology, University of Manitoba, Winnipeg, Man.

² Professor of Zoology, University of Manitoba.

No reference will be made to questions of technique as an account of the principal methods used in the study of cestode material has recently been published by the writer (63).

Taxonomy

Class CESTODARIA Monticelli, 1892

Of the two families which comprise this class of Helminthes, the *Amphilinidae* are not represented in Canadian records, and the *Gyrocotylidae* are represented by one species only. No comprehensive monograph on the class is available, but a taxonomic survey of the genus *Gyrocotyle* has been published by Dollfus (12), and gyrocotylid material from Californian waters has been described by Kofoed and Watson (26), by Watson (67) and by Ward (58).

Fam. GYROCOTYLIDAE Benham, 1901

Gyrocotyle urna Grube et Wagener, 1852. Not uncommon in the intestine of *Hydrolagus colliei* in the Straits of Georgia, B.C. Usually two twin individuals are present in each infested host. *Gyrocotyle fimbriata* Watson, 1911, appears to be synonymous with this species. *Reference*: Wardle (60).

Class CESTODA s. str.

Superfam. DIBOTHRIOCEPHALOIDEA Stiles, 1906

Although the generic term *Dibothriocephalus* Luhe, 1899, is now generally superseded in nomenclature by *Diphyllbothrium* Cobbold, 1858, there is still some doubt as to whether the two genera are synonymous and the point can only be decided by a re-examination of material from the dolphin species from which Cobbold obtained his *Diphyllbothrium stemmacephalum*. If they are synonymous, the superfamily designation should of course be changed to Diphyllbothrioidea but in the meantime the term originally suggested by Stiles may be allowed to stand.

There is no comprehensive publication dealing with the whole superfamily. The families Caryophyllaeidae, Cyathocephalidae, and Amphicotylidae have been partially monographed by Nybelin (44); the Caryophyllaeidae have also been monographed by Hunter (24). Keys to the species of the genus *Diphyllbothrium* have been provided by Meggitt (37) and Baylis (2), and the biology of *D. latum* in North America has been discussed by Essex (17), Magath (33, 34), Vergeer (57), Ward (59) and others, in addition to the Canadian references given. The dibothriocephaloid tapeworms of eastern Canada and of Arctic Canada have been recorded by Cooper (6-11); those of western and northern Canada by Wardle (60, 61, 62, 64). Of the eight families constituting the superfamily, all, except the Echinophallidae, are represented by one or more species in the area.

Fam. CARYOPHYLLAEIDAE Müller, 1787

Glaridacris catostomi Cooper, 1920. Common in the intestine of species of *Catostomus*, probably over the whole area; adults and larvae may occur in the same individual host, the adults free in the gut lumen, the larvae imbedded in pits in the mucosa. Recorded from *Catostomus catostomi*, Lake Waskesiu, Sask., and Lake Simcoe, Ont. *Reference*: Wardle (61).

Fam. CYATHOCEPHALIDAE Lühe, 1899

Diplocotyle olrikii Krabbe, 1874. Common and numerous in the intestine of *Salvelinus alpinus stagnalis*, *Salvelinus fontinalis*, *Leucichthys artedi*, *Coregonus clupeaformis*, *Coregonus atikameg*, *Myoxocephalus quadricornis*, in Hudson Bay, Wakeham Bay, Churchill River, James Bay. In *Salvelinus malma*, Bernard Harbor, N.W.T.

It appears to be typically a parasite of circum-arctic Salmonoidea, but has been recorded in *Pseudopleuronectes americanus* at St. Andrews, N.B. The species *Bothrimonus intermedius* Cooper and *Diplocotyle nylandica* Schneider are in the writer's opinion merely synonyms of *D. olrikii*. *References*: Cooper (9, 11), Wardle (61).

Cyathocephalus truncatus (Pallas, 1781). Not uncommon in the intestine of fresh-water salmonoids, probably over the whole area east of the Rocky Mountains. Recorded from *Coregonus clupeaformis* and *Leucichthys zenithicus*, Lake Winnipeg; from *Salvelinus alpinus malma*, Spray Lakes, Alberta; from *Cristivomer namaycush*, Bernard Harbor, N.W.T.; and from *Coregonus clupeaformis*, Lake Ontario. The species *C. americanus* Cooper appears to be synonymous with *truncatus*. The plerocercoid larva occurs in the amphipod species *Pontoporeia affinis* (Lindström). *References*: Cooper (9, 11), Wardle (61).

Fam. DIBOTHRIOCEPHALIDAE Lühe, 1902

Subfam. 1. LIGULINAE Lühe, 1899

Ligula intestinalis (Linn. 1758). Common as a plerocercoid larva in the body cavity of fresh-water fishes across the area. The writer has specimens from *Catostomus*, *Notropis*, *Micropterus*, *Gasterosteus*, *Salmo*, *Perca*, varying in length from 20 to 600 mm. The adult has been recorded in Canada only from *Mergus americanus*, Shuswap Lake, B.C., and in the United States only from *Mergus* sp. and *Mergus merganser*, although reported in Europe from a wide range of water birds. *References*: Cooper (9), Wardle (62).

Schistocephalus solidus (Müller, 1776). Not uncommon as a plerocercoid larva in the body cavity of fresh-water fishes. It has been thus reported in *Cottus* sp., Shuswap Lake, B.C.; *Uranidea formosa*, Port Credit, Ont.; *Gasterosteus bispinosus*, Chamcook Lake, N.B.; *Pygosteus pungitius*, Bernard Harbor, N.W.T. The writer has also examined material from *Salvelinus fontinalis*, in Big Bent, Pumpkin Seed and Commandant lakes, Quebec.

The adult has been recorded in Canada only from *Mergus americanus*, Nanaimo, B.C., and as lying free on the shore of Nanaimo Lake, B.C. In the United States it has been recorded only in *Lophodytes cucullatus*. *References*: Cooper (9, 11), Wardle (62).

Subfam. 2. DIBOTHRIOCEPHALINAE Lühe, 1902

Diphyllbothrium latum (Linnaeus, 1758). Common but localized in distribution, in man, dog, bear, mink, cat, in Manitoba, possibly in Saskatchewan. Recorded in 81% of 500 dogs in the vicinity of Lake Winnipeg in 1931; in 4% of vagrant cats in Winnipeg in 1930-31. Proceroid larva in *Diaptomus oregonensis*. Plerocercoid larva in epaxonic and hypaxonic musculature, and, rarely, on peritoneal gut surface, of *Esox lucius*, *Lucioperca vitreum*, *Perca flavescens*, *Lucioperca canadense*; mean number of larvae per fish ranges from 2.3 in *Esox* to 1.2 in *L. canadense*. The adult worm agrees closely in structural features with European and Japanese material of *D. latum* and it seems well established that Canadian material is either this form, imported from Europe, or an indigenous subspecies identical morphologically with it. *References*: Nicholson (41, 43), Wardle (61, 64).

Diphyllbothrium canadense Cooper, 1921. Recorded from intestine of Northern Raven (*Corvus corax principalis*) at Bernard Harbor, N.W.T. Possibly synonymous with *D. cordiceps* Weinland, recorded by Linton (29) from *Pelicanus erythrorhynchus*, Yellowstone Lake, Wyoming. *Reference*: Cooper (11).

Diphyllbothrium cordatum (Leuckart, 1863). Recorded from the bearded seal (*Erignathus barbatus*) at Bernard Harbor, N.W.T., together with ***Diphyllbothrium lanceolatum*** (Krabbe, 1865) and ***Pyramicocephalus phocorum*** (Fabricius, 1780). The larval form of the latter species has been recorded by the writer from *Gadus ogac*, Richmond Gulf, Hudson Bay. *References*: Cooper (11), Wardle (61).

***Diphyllbothrium* sp. larvae inquirendae.** The term is used to imply larvae to which the crucial test of rearing in a suspected adult stage host has not been applied, so that they cannot be definitely assigned to any known species. Seven such diphyllbothriid larvae are known from Canadian hosts, namely:—

1. Free in gut of *Salvelinus marstoni*, Bernard Harbor, N.W.T. *Reference*: Cooper (11).

2. Free in gut of *Erignathus barbatus*, Bernard Harbor, N.W.T. Possibly the larva of *D. lanceolatum* (Krabbe, 1865). *Reference*: Cooper (11).

3. Free or encysted on the peritoneal gut surface and coelomic surface of *Oncorhynchus nerka kennerlyi* in Kootenay and Chilliwack lakes, B.C. Common and numerous. Possibly identical with the form described by Fasten (19) from Cutthroat trout in Klause and Cooper lakes, Washington, and from *Oncorhynchus tshawytscha* and *O. nerka kennerlyi* in Klause Lake, Washington. *Reference*: Wardle (60).

4. Free on peritoneal gut surface of *Oncochynchus kisutch*, Straits of Georgia, B.C. Possibly identical with the form described by Linton (29) in *Salmo mykiss*, Yellowstone Lake, Wyoming, as the larva of *D. cordiceps*. *Reference*: Wardle (60).
5. Encysted in liver of *Lota lota maculosa*, Lake Winnipeg; uncommon. *Reference*: Wardle (61).
6. Free or encysted on the peritoneal gut surface of *Salvelinus fontinalis*, Wakeham Bay, Ungava. Numerous in host. *Reference*: Wardle (61).
7. Embedded in liver of fingerling *Salvelinus fontinalis*, Yarmouth, N.S., and inducing abdominal ascites; one larva per host. No previous record.

Fam. PTYCHOBOTHRIDAE Lühe, 1902

Bothriocephalus scorpii (Müller, 1776). Not uncommon in the pyloric caeca of shore-frequenting teleostean fishes in Atlantic and Pacific coastal waters of the area. It has been recorded from *Hemitripterus americanus*, St. Andrews, N.B.; from *Myoxocephalus quadricornis*, Bernard Harbor, N.W.T.; from *Leptocottus armatus*, *Enophrys bison*, *Hexagrammos decagrammus*, *Hexagrammos superciliosus*, *Myoxocephalus polycanthocephalus*, *Apodichthys flavidus*, Nanaimo, B.C. *References*: Cooper (9, 11), Wardle (60).

Bothriocephalus occidentalis (Linton, 1898). Recorded from the pyloric caeca of *Leptocottus armatus* and *Sebastodes* spp., Nanaimo, B.C. Uncommon. *Reference*: Wardle (60).

Bothriocephalus cuspidatus Cooper, 1921. Common in the gut of fresh-water teleosts over the whole area east of the Rockies. It comprises probably a group of subspecies, such as *B. c. cuspidatus* in *Lucioperca vitreum*, *B. c. hiodontos* in *Hiodon* spp. and *B. c. luciopercae* in *Lucioperca canadense*. The life history in Minnesota waters has been elucidated by Essex (18). *References*: Cooper (8, 9), Wardle (61).

Bothriocephalus claviceps (Goeze, 1782). Recorded in *Anguilla rostrata*, Chamcook Lake, N.B. *Reference*: Cooper (9).

Cleistobothrium crassiceps (Rudolphi, 1819). Common and numerous in *Merluccius bilinearis*, St. Andrews, N.B., and *Merluccius productus*, Nanaimo, B.C. *References*: Cooper (9), Wardle (62).

Fam. TRIAENOPHORIDAE Nybelin, 1922

Triaenophorus tricuspidatus (Bloch, 1779). Common in gut of *Esox lucius* over the whole area. Plerocercoid larvae encysted in musculature of coregonid fishes and in the liver of young *Esox* and *Perca*. The species *T. nodulosus* (Pallas) and *T. robustus* (Olsson) are regarded by the writer as synonyms of *tricuspidatus*. *References*: Cooper (9), Nicholson (42), Newton (40), Wardle (61).

Fam. AMPHICOTYLIDAE Nybelin, 1922

Subfam. 1. AMPHICOTYLINAE Lühe, 1902

Eubothrium oncorhynchi Wardle, 1932. Not uncommon in the pyloric caeca of *Oncorhynchus* spp. Straits of Georgia, B.C.; 3–10% of fishes are infected during the summer months. Usually one strobila per host, occasionally two, rarely more. *Reference*: Wardle (60).

Eubothrium crassum (Bloch, 1779). Recorded in pyloric caeca of *Salvelinus alpinus stagnalis*, Hudson Bay. Common and numerous. In intestine of *Myoxocephalus quadricornis*, Churchill River; in *Salmo salar*, Miramichi River, N.B. Common. *References*: Wardle (61), Kuitunen-Ekbaum (27).

Eubothrium salvelini (Schränk, 1781). Recorded in the pyloric caeca of *Salvelinus fontinalis*, James Bay; *Salvelinus alpinus malma*, Spray Lakes, Alberta; *Cristivomer namaycush*, Clear Lake, Manitoba, Lake Manitoba, Lake Huron, Lake Temagami, Lake Ontario. Probably coexistent with *Salvelinus* and *Cristivomer* across the area. *References*: Cooper (9), Wardle (61), Kuitunen-Ekbaum (27).

Eubothrium rugosum (Batsch, 1786). Common in the pyloric caeca of *Lota lota maculosa*, lakes Winnipeg, Huron, Ontario. Dimorphic, with *conformatus* type and *deformatus* type. *References*: Cooper (9), Wardle (61, 65), Kuitunen-Ekbaum (27).

Subfam. ABOTHRIINAE Nybelin, 1922

Abothrium gadi Van Beneden, 1871. Intestine of *Melanogrammus aeglefinus*, Passamaquoddy Bay, N.B. and Bay of Fundy, N.S., *Gadus callarias*, Campobello Island, N.B. *Reference*: Cooper (9).

Fam. HAPLOBOTHRIIDAE Cooper, 1914

Haplobothrium globuliforme Cooper, 1914. Recorded from *Amia calva* at Go-Home Bay, Muskoka, Ont. *References*: Cooper (6, 9).

Superfam. TETRARHYNCHOIDEA Southwell, 1930

No comprehensive monograph on this superfamily has yet been published and the classification is chaotic. Reference may be made to the publications of Dollfus (13, 14, 15), Guiart (21, 22), Pintner (45–48) and Southwell (52, 53). The classification adopted here is a modification of that suggested by Guiart which has the disadvantage that it is based primarily upon larval characters, so that any adult form whose larval stage is unknown, must necessarily remain *incertae sedis*. The Canadian tetrarhynchoids have been completely neglected. Linton (30) has described however from the adjacent New England waters, *Otobothrium crenacolle* and a number of created species of the now discredited genera *Rhyncobothrium*, *Synbothrium* and *Tetrarhynchus*.

Fam. SPHYRIOCEPHALIDAE Guiart, 1927

No Canadian records.

Fam. DIBOTHRIORHYNCHIDAE Dollfus, 1931

Dibothriorhynchus grossus (Rudolphi, 1810), *larva inquirenda*. Recorded from body cavity of *Gadus callarias*, Port Burwell, Ungava. *Reference*: Wardle (61).

Fam. TENTACULARIIDAE Dollfus, 1931

Nybelinia surmenicola Okada, *larva inquirenda*. Common and numerous in the stomach of *Ophiodon elongatus*, and the epaxonic musculature of *Theragra* spp., Straits of Georgia, B.C. *References*: Wardle (60, 66).

Fam. VAULLEGARDIDAE Guiart, 1927

No Canadian records.

Fam. LAKISTORHYNCHIDAE Guiart, 1927

Grillotia erinacea (Van Beneden, 1857). Recorded as adult in the intestine of *Ilexanchnus caurinus*, Straits of Georgia, B.C., and as a larva in the musculature of *Theragra* spp., same locality. *Reference*: Wardle (62).

Fam. EUTETRARIHYNCHIDAE Guiart, 1927

No Canadian records.

INCERTAE SEDIS

Gilquinia squali (Fabricius, 1793). Common and numerous in the intestine of *Squalus sucklii*, Straits of Georgia, B.C. *Reference*: Wardle (60).

Superfam. PHYLLOBOTHRIOIDEA Southwell, 1930

This superfamily has been partially monographed by Southwell (50). Of the two families recognized by this author, the ***Oncobothriidae*** are not as yet represented in Canadian records, and the ***Phyllobothriidae*** are represented by a single larval form, probably in an abnormal host. Linton (30), however, has recorded 10 phyllobothriid and 13 oncobothriid species from fishes in the adjacent coastal waters of New England, and there is little doubt that a survey of selachian fishes in Canadian waters would yield a number of phyllobothrioid forms.

Fam. PHYLLOBOTHRIDAE Braun, 1900

Phyllobothrium salmonis Fujita, 1922, *larva inquirenda*. Common in either encysted or free condition in the alimentary tract of *Oncorhynchus* spp., especially *O. gorbuscha* in British Columbia coastal waters. The adult has been recorded under the name of *Phyllobothrium keta* by Canavan (5) from a specimen of *O. keta* in Alaskan waters. *Reference*: Wardle (60).

Superfam. LECANICEPHALOIDEA Southwell, 1930

Fam. LECANICEPHALIDAE Braun, 1900

No Canadian representatives of this family have been recorded but Linton (30) has recorded *Discocephalum pileatum* in species of *Carcharinus* in New England waters.

Superfam. PROTEOCEPHALOIDEA Southwell, 1930

The North American species of this family have been monographed, up to date of publication, by La Rue (28), and a criticism of the characters used in distinguishing proteocephaloid genera and species, with a key to the species known at the date of publication, has been published by Meggitt (38). The known Canadian species belong to the genus *Proteocephalus*, Weinland, 1858 (= *Ichthyotaenia* Lonnberg, 1894) and to the genus *Corallobothrium* Fritsch, 1866. No members of the family Monticellidae have been recorded in the area.

Fam. PROTEOCEPHALIDAE La Rue, 1911 (= *Ichthyotaeniidae* Ariola, 1899)

Proteocephalus coregoni Wardle, 1932. Recorded in intestine of *Coregonus atikameg*, Hudson Bay. Reference: Wardle (61).

Proteocephalus laruei Faust, 1920. Common and numerous in the intestine of *Coregonus clupeaformis* and *Leucichthys xenithicus*, Lakes Winnipeg and Waskesiu; in *Leucichthys hoyi*, Lake Winnipeg; in *Coregonus clupeaformis*, Lake Ontario. The common proteocephalid of coregonid fishes in the area. Reference: Wardle (61).

Proteocephalus pinguis La Rue, 1911. Recorded in the intestine of *Esox lucius*, Lakes Winnipeg and Winnipegosis. The common proteocephalid of *Esox* in the area. Reference: Wardle (61).

Proteocephalus arcticus Cooper, 1921. Recorded from *Salvelinus marstoni*, Bernard Harbor, N.W.T.; from *Salmo clarkiei* fingerlings, Departure Bay creek, Vancouver Island; from *Oncorhynchus kisutch* fingerlings, Cultus Lake, B.C. References: Cooper (11), Wardle (62).

Proteocephalus ambloplitis (Leidy, 1887). Recorded by Cooper as adult in the intestine of *Micropterus dolomieu*, and as larval in the body cavity of the same host, Georgian Bay, Lake Huron; noted by the writer in the intestine of *M. dolomieu*, Whitefish Lake, Quebec, both in adult and larval stages; and as a larval stage only in *Ameiurus nebulosus*, same locality. Reference: Cooper (7).

Corallobothrium fimbriatum Essex, 1927, *larva inquirenda*. Common in the intestine of *Ameiurus nebulosus*, Lake Winnipeg, about six larvae per host. Reference: Wardle (61).

Superfam. TAENIOIDEA Zwicke, 1841

Eleven families of taenioid cestodes are recognized by Southwell (53), and to these may be added the families *Diploposthidae* Poche and *Biuterinidae* Meggitt. Representatives of only seven of these families have been recorded in the area, and the forms recorded here bring the total number of recorded Canadian taenioids from 11 to 28. There can be little doubt that this meagre total is not fully representative of the distribution of this superfamily in the area and that the forms recorded in domesticated mammals and birds, and in many of the migratory wild birds, in the United States will eventually be found also in Canada.

The taenioid species in the United States have been recorded by Stiles and Hassall (56), Stiles (55), Ransom (49), Hall (23), Mayhew (35), Douthitt (16), Linton (31), Millzner (39) and others.

Fam. TAENIIDAE Ludwig, 1886

Multiceps multiceps (Leske, 1780). Recorded as isolated proglottides from the dogs of the Canadian Arctic Expedition, 1913-18, at Collinson Point, Alaska. *Reference*: Cooper (11).

Multiceps serialis (Gervais, 1847), *larva inquirenda*. Common in *Lepus americanus* in western Canada in the form of cysts, $\frac{1}{2}$ -2 $\frac{1}{2}$ in. in diameter, in the subcutaneous tissue, each cyst having several hundred scolices. Percentage of infestation among 400 rabbits in Manitoba was 15.6 in 1931. The maximum number of cysts per host was 14. *Reference*: Boughton (3).

Taenia hydatigena (Pallas, 1776). Found by Riddle (unpub.) in 4 out of 70 vagrant cats in Winnipeg, 1931-32. To this species the writer would refer also a *larva inquirenda* common in the muscles of *Alces alces* in Alberta.

Taenia pisiformis (Bloch, 1780), *larva inquirenda*. Common as transparent vesicles in the liver and coelom of *Lepus americanus*, Manitoba and Saskatchewan. Boughton records an incidence of 14.7% in 400 rabbits from Manitoba, 1931, the average number of cysts per host being 20, the maximum number per host being 106. *Reference*: Boughton (3).

Taenia solium (Linnaeus, 1758). There are apparently no records of this cosmopolitan parasite of man in Canada, although there is no doubt of its occurrence in the area. The writer is informed by veterinary surgeons that the larva is much less common in hogs than was formerly the case. The writer has material from Saskatchewan hogs.

Taeniarhynchus saginatus (Goeze, 1782). There appear to be no published records of this form in the area but the writer has larval material from western Canadian cattle. It is probably a common parasite of man in the area.

Fam. ANOPLOCEPHALIDAE Cholodkowsky, 1902

The North American species of this family, recorded prior to 1915, have been described by Douthitt (16). The European forms have been mono-

graphed by Baer (1). Of the four subfamilies recognized by Douthitt, the Linstowinae and Avitellinae have not been recorded in the area.

Subfam. ANOPLOCEPHALINAE Blanchard, 1891

Cittotaenia pectinata americana Douthitt, 1915. Common in young *Lepus americanus* in Manitoba. Boughton records 100% of infestation in rabbits 3–8 weeks old between June and November but practically no infestation after the beginning of November, when the animals commence to feed largely upon bark, and an infestation of only 0.26% in rabbits more than one year old. *Reference*: Boughton (3).

Moniezia expansa (Rudolphi, 1805), ***Moniezia alba*** (Perroncito, 1897), ***Moniezia planissima*** (Stiles and Hassall, 1893). Described as common in lambs in eastern Canada. *Reference*: Stevenson (54).

Subfam. THYSANOMINAE Fuhrmann, 1907

Thysanosoma actinioides Dicsing, 1834. Described as occurring in the bile ducts of lambs in eastern Canada. *Reference*: Stevenson (54).

Fam. DAVAINIIDAE Fuhrmann, 1907

Davainea comitata Ransom, 1909. Recorded from Yellow-bellied Sapsucker (*Sphyrapicus varius varius*), Tamaracouta, Quebec, in the abdominal cavity. *Reference*: Lloyd (32).

Fam. HYMENOLEPIDIDAE Railliet et Henry, 1909

A monographic study of the North American species of this large family has been published by Mayhew (35). Reference may be made also to the publications of Ransom (49) and Linton (31). Of the five subfamilies recognized by Mayhew, the *Oligorchinae* and *Diorchinae* are not represented in Canadian records.

Subfam. HYMENOLEPIDINAE Ransom, 1909

Weinlandia planestici Mayhew, 1925. Occurring in intestine of *Planesticus migratorius*, Saskatoon. This, and *W. microcirrosa* are probably widely distributed over western Canada in the American robin.

Weinlandia corvi Mayhew, 1925. In *Corvus brachyrhynchos hesperis*, Saskatchewan. Common. Three specimens per host.

Subfam. FIMBRIARINAE Fröhlich, 1802

Fimbriaria intermedia Fuhrmann, 1903. Recorded from intestine of the Pacific Eider-duck (*Somateria v-nigra*) at Bernard Harbor, N.W.T. *Reference*: Cooper (11).

Fimbriaria fasciolaria (Pallas, 1781). The writer has material from *Mergus americanus*, Nanaimo, B.C. It has not previously been recorded in the area though probably widespread and common. Linton (31) reports it in *Mergus serrator*, on the New England coast.

Subfam. APLOPARAKSINAE Mayhew, 1925

Aploparaksis sp. Recorded from *Somateria v-nigra*, at Bernard Harbor, N.W.T. Reference: Cooper (11).

Fam. DILEPIDIDAE Railliet et Henry, 1909

This large family of bird-infesting cestodes is probably more widely distributed in the area than appears to be indicated by the ten species recorded here.

Subfam. DILEPIDINAE Railliet et Henry, 1909

Lateriporus geographicus Cooper, 1921. Recorded as present in stomach of *Somateria v-nigra*, Bernard Harbor, N.W.T. Reference: Cooper (11).

Lateriporus sp. To this genus the writer would refer a collection of small cestodes, too fragmentary to identify specifically, taken from the intestine of a Dipper (*Cinclus mexicanus*) at Taft, B.C.

Anomotaenia constricta (Molin, 1858). Common and numerous in intestine of *Corvus brachyrhynchos brachyrhynchos*, in Manitoba.

Anomotaenia sp. The writer would refer to this genus a number of small cestodes from the Willett (*Catoptrophorus semipalmatus*), Saskatoon, which, owing to the partial obliteration of the rostellar armature, cannot be specifically identified. They are cestodes up to 100×1.5 mm. in dimensions with segments campanulate when mature, to longi-rectangular when gravid, in shape, and markedly salient. The scolex is approximately 0.3 mm. in length and its rostellum has one circle of hooks. The testes lie completely behind the germarium and vitellarium and number 36. The genital pores are alternate and marginal, one-fifth of the proglottid length from the anterior border. The eggs occur in packets of 4-8 apparently in the parenchyma.

Subfam. DIPYLIDINAE Stiles, 1896

Dipylidium caninum (Linnaeus, 1758); ***Dipylidium sexcoronatum*** Ratz, 1900; ***Dipylidium compactum*** Milzner, 1926; ***Dipylidium gracilis*** Milzner, 1926; ***Dipylidium diffusum*** Milzner, 1926. All found by Riddle (unpub.) in *Felis domesticus*, Winnipeg. Common. Numerous. Incidence of infestation among 72 cats was 25%.

Subfam. PARUTERININAE Ransom, 1909

Anonchotaenia sp. The writer would refer doubtfully to this genus two immature and damaged cestodes from *Dendroica aestiva*, Saskatoon. Ransom (49) has recorded *A. globata* from *Dendroica striata*.

Fam. ACOLEIDAE Ransom, 1909

The writer would refer to this family some fragments of a broad, fleshy cestode obtained from *Limnodromus griseus*, Alberta, which, although lacking a scolex, is undoubtedly acoleid in morphology. It may be noted that *Acoleus vaginalis* (Rudolphi, 1819) is recorded by Ransom (49) in the related host species *Himantopus mexicanus*.

Fam. TETRABOTHRIIDAE Linton, 1891

Tetrabothrius cylindraceus (Rudolphi, 1819). Common in intestine of *Larus argentatus*, Nanaimo and Deep Bay, Vancouver Island.

Discussion

Of the 53 species of Cestoda reported in the foregoing pages, 6 are at present known only in Canadian hosts; of the remaining species, 29 occur also in northwestern Europe, 14 are recorded elsewhere only in the United States, and 4—the ones referable to genus only—might be either European or United States in co-distribution. It may be noted that the six Canadian forms belong to genera occurring in Europe and are specifically very similar to European species. Of the 24 larval cestodes reported, 14 and one doubtful form are also European, 3 and two doubtful forms occur also in the United States, 2 occur also in Japanese waters, and 2 are unassignable.

The species which are coincident with European species appear to be more restricted in host distribution in North America than in Europe. While this may be more apparent than real, since fewer host species have been examined in this area, yet it appears to hold good for those species which, even in Canada, have been very fully studied. *Diphyllobothrium latum*, for example, has never been recorded in North America from salmonoid fishes, in striking contrast to the host distribution in Europe and Japan; *Triaenophorus tricuspidatus* in Canada is restricted in larval distribution to coregonid fishes whereas in Europe it has been recorded in pike, perch, ling, trout, grayling, catfish, popefish, salmon, etc.; *Cyathocephalus truncatus*, as an adult, is more restricted in host species in North America than in Europe and both adults and larvae of *Ligula intestinalis* and *Schistocephalus solidus* are similarly more restricted. Further it may be noted that North American forms which coincide with European species, show commonly certain morphological differences, not always sufficient to justify specific separation, although there is a tendency among North American workers towards such separation.

The inference might be drawn, therefore, although it is weakened by the paucity of information concerning North American cestodes, that North America has received most of its cestode species from Eurasia.

The genus *Proteocephalus*, however, seems definitely more American in possible origin than European. Of 44 species distinguished by characters other than dimensional ones, and reviewed by Meggitt (38), 19 are Old World in distribution, 25 are North American.

In any evaluation of the economic status of the Cestoda of the area, there are two viewpoints to be considered; namely, (1) the influence of cestode infestation upon the health of the host, and (2) the influence of such infestation upon the commercial value of the host.

Although possible injury to the host may be produced by a cestode in many ways—by occlusion of the gut lumen, by abstraction of the products of digestion, by intoxication of the host tissues with excretory products, by pressure upon a physiologically important organ—yet actual proved cases of such injury are few. Admittedly the pathology of wild animals remains almost completely unstudied and many cases of physiological injury to a host animal might pass unnoticed, but so far as parasitological experience goes, there is no evidence that enteric infestation with cestode parasites is seriously inimical to a *mature* animal. The intestine of fresh-water fishes is commonly so blocked with strobilae as to occasion surprise that the animals can gain any advantage whatever from their alimentary functions, yet in the examination of many thousand such fishes the writer cannot recall a single case of obvious malnutrition in a mature fish attributable to cestode infestation.

On the other hand, there is considerable evidence to show that such infestation may provoke malnutrition and nervous disturbance in an *immature* animal. There is considerable epidemic mortality in Canadian hatcheries among fingerling trout and salmon, attributable to gut occlusion with such cestodes as *Cyathocephalus* and *Eubothrium*, and such victims show all the external signs of malnutrition.

Nor can the common textbook suggestion that host animals may be injured by toxic excretions from cestode parasites, be supported by known facts. *Diphyllobothrium latum* is commonly asserted to be provocative of pernicious anaemia in the human host but Magath (33) from an analysis of a large number of such cases in the Mayo Clinic is disposed to deny any causal relation between tapeworm infestation and primary anaemia. It must be emphasized that there is no experimental evidence in favor of the possibility of adult cestoda excreting haemolytic waste products. The known facts concerning cestode physiology are scanty in the extreme but such information as is available suggests that the primary nutritive requirement of the cestode is glucose and that the cestode excretions are such as would be produced by glucose degradation. Brand (4) has demonstrated in the case of *Moniezia expansa*, that carbon dioxide, succinic acid, lactic acid and higher fatty acids are excreted into an artificial medium such as Ringer's solution, under anerobic conditions and at mammalian body temperature. There is no reason to regard such products as particularly toxic to a host animal, and the inimical effect of cestode infestation upon growing animals would appear to be due to the direct abstraction of the products of digestion or direct interference with the liver functions, rather than to intoxication by excretory products.

There is however a considerable mass of evidence to support the view that larval cestodes, somatic rather than enteric in host location, may bring

about serious disturbance by direct interference with the physiological efficiency of the organs in which they are located. Well-known and well-established examples of such pathogenicity are of course provided by the larval stages of *Echinococcus granulosus* in man, the so-called "hydatid cysts"; by the provocation of hepatic sarcoma in rats by the larva of *Taenia fasciolaris*; the inhibition of egg production in *Micropterus dolomieu* by larval *Proteocephalus ambloplitis*; the clogging of the atrium of the heart, or of the large blood sinuses of coregonid fishes by larval *Schistocephalus solidus*; the pathological changes in the gastric mucosa noted by the writer (Wardle, 62) in the Pacific fish *Ophiodon elongatus* when infested heavily with larval *Nybelinia surmenicola*; the abdominal dropsy and associated mortality of fingerling salmonid fishes in eastern Canadian hatcheries, when infested with a diphyllbothriid larva embedded in the liver tissue.

On the other hand, fresh-water fishes intensely infested with intermuscular larvae of *Diphyllbothrium latum* or *Triaenophorus tricuspidatus* do not seem unduly inconvenienced nor do they show any external morphological characteristics of such infestation. Boughton (3) was unable to associate the intense infestation of *Lepus americanus* by larval *Taenia pisiformis* and *Multiceps serialis* with the fluctuations in abundance of this host animal.

Although there is no reason to regard cestode infestation as particularly dangerous to human health, there is naturally somewhat of a prejudice towards foodstuffs which are infested with larval cestodes, and the influence of such infestation upon the commercial value of the infested animal is by no means negligible.

Johnstone (25), in reference to the seizure of marketable marine fishes by food inspectors at Liverpool, owing to their infestation with larval *Grillotia erinacea*, asserts that although in this case such infestation is harmless to the human consumer "the inspectors probably acted in the interests of public health in condemning such articles of food as contained obvious cyst-like structures in the flesh, as to the precise nature of which they were ignorant, since there is the possibility that these bodies might be detrimental to the health of those eating them. Further, the presence of these cysts was very obvious and rather unpleasant to the eye and might easily have prejudiced a customer against a particular vendor or fish".

A similar but much more serious example of the commercial importance of what might be called "aesthetic prejudice" is afforded by the infestation of coregonid fishes, especially the genus *Leucichthys*, in North America, by the larval stages of *Triaenophorus tricuspidatus*. The adult worm appears specific to *Esox*, possibly to *Cristivomer* and is therefore most unlikely to be capable of establishment in a warm-blooded host. Experimental tests by the writer suggest convincingly that this cestode cannot develop in the dog or in man and that it cannot withstand for more than a brief period, either the mammalian range of body temperature or the concentration of mammalian gastric juice. Nevertheless the rigid inspection tests imposed by the United States health authorities upon Canadian coregonids intended for consumption

in the United States—whilst unjustifiable upon scientific grounds and unfortunate in their inimical effect upon a thriving fresh-water fishing industry—cannot be severely criticized from the standpoint of public health, except as erring on the side of severity, since there is no question that a cisco heavily infested with the large, yellow bladders of the larval cestode, which when injured exude an unpleasant pus-like fluid, is not only most objectionable aesthetically but is most likely to prejudice the consumer against any kind of Canadian fish.

The redfish or Kokanee (*Oncorhynchus nerka kennerlyi*) of certain lakes in British Columbia is commonly so heavily infested with diphylobothriid larvae that if this fish were of commercial value its sale on the public market would not be tolerated by any competent public health authority, although it is extremely probable that the infesting larvae can only develop further in certain water birds. It is in fact fortunate for the extensive salmon fishing industries that this infestation is restricted to the landlocked variety of Pacific salmon and does not occur in the marine species.

The aesthetic aspect of helminth infestation, the natural human prejudice against food infestation by "worms" is in the writer's opinion a factor far more important in the economic evaluation of the cestodes of an area than is the somewhat remote possibility of an animal being infested with some helminth that is directly communicable to man.

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TETRAMERES CRAMI SP. NOV., A NEMATODE PARASITIZING THE PROVENTRICULUS OF A DOMESTIC DUCK IN CANADA¹

By W. E. SWALES²

Abstract

A duck obtained from a farm near Ottawa, Canada, was found on post-mortem examination to be parasitized by a hitherto unreported nematode. No males were discovered but an examination of the nine females obtained revealed certain characteristics which appeared to justify the formation of a new species, for which the name *Tetrameres crami* is proposed.

In April, 1932, a duck obtained from a farm in the vicinity of Ottawa, Ontario, Canada, and used for a short period for experimental purposes at the Animal Diseases Research Institute, Hull, Quebec, was found on post-mortem examination to be parasitized by a hitherto unreported nematode.

The walls of the proventriculus were found to contain several small dark objects which proved, on dissection, to be nematodes deeply embedded in the crypts of Lieberkühn. In this way, nine examples were obtained.

Examination of the live specimens showed that they were members of the genus *Tetrameres* Creplin, 1846. Unfortunately, in spite of a careful search, no males were discovered.

The worms were fixed and preserved at the time of collection but a taxonomic study was delayed until recently, when through the courtesy of Dr. E. A. Watson, the Director of the Animal Diseases Research Institute, the helminthological collection from that laboratory was placed at the disposal of this Institute.

A study of the females thus collected revealed certain characteristics which, in spite of the absence of males, appeared to justify the formation of a new species. For this, the name *Tetrameres crami* is proposed in recognition of the work of Dr. Eloise Cram, which has so greatly facilitated the study of avian parasites in North America.

Tetrameres crami, sp. nov.

Male. Unknown.

Female. Body globular to sub-globular, 1.5 mm. to 2.3 mm. long by 1.2 mm. to 1.7 mm. wide, strongly furrowed longitudinally, the furrows corresponding to the median and lateral lines. The cuticle is also strongly striated transversely. The cephalic and caudal extremities protrude from the body mass; each is conical in outline.

The buccal capsule is almost circular, measuring 14 μ long by 12 μ wide, and is strongly cuticularized. The muscular oesophagus is well developed

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but is shorter than in the other members of the genus, being 0.16 mm. to 0.18 mm. long. It is surrounded near its middle by a prominent nerve ring, immediately behind which is situated the large excretory pore. Only one cervical papilla was seen in the type specimen, situated 72 μ from the anterior extremity. The oesophagus is nearly a millimetre long, penetrating the body mass to join the large sacular intestine which, owing to its distention with detritus, is seen as a dark irregular mass. The rectum is well defined and is 75 μ long; the anus is 0.15 mm. from the tip of the tail.

The vulva is 0.25 mm. from the tip of the tail and the ovejector is large with a somewhat barrel-shaped terminal portion, 40 μ long by 29 μ wide, with a simple small vestibule; there is no copulatory receptaculum present. The uteri and ovarian tubules are very long, their coils practically filling the body cavity. The eggs are thick-shelled and oval, measuring 45 to 48 μ long by 16 to 18 μ wide.

Remarks. This species differs in many respects from the other known North American species, *T. americana* Cram, 1927, being more closely related to *T. coccinea* (Seurat, 1914) and *T. cochleariae* Travassos, 1917. It differs from the former chiefly in the length of the muscular oesophagus, the relative positions of the anus and vulva, and in the dimensions and form of the ovejector. In comparison to the latter, it is smaller in almost every respect.

Hosts. *Primary:* *Anas boschas domestica*.

Secondary: Unknown, probably the same as that of *T. fissispina*, *Daphnia pulex* being very common in the locality.

Location. Proventriculus, the females embedded in the crypts of Lieberkühn, the males probably in the lumen.

Distribution. Canada (Ottawa, Ontario).

This is the first record of a member of the Family Tetrameridae being found in Canada, and according to Cram (2) American reports probably deal wholly with *T. americana*.

The Muscular Oesophagus of Tetrameres

It will be noted that in the above description the anterior portion of the oesophagus was not described as a pharynx, as it has been in other descriptions of related species. A pharynx, as defined by Baylis and Daubney (1), is an organ resembling a buccal capsule but having an exterior muscular coat. The anterior portion of the oesophagus in *T. crami*, *T. americana* and other species cannot be said to resemble a buccal capsule and the strongly muscular structure in two species examined is apparently interspersed with glandular tissue.

Although Travassos (6) in his illustration of *T. fissispina* does not show the nerve ring, his measurements indicate that it surrounds the so-called pharynx approximately at its middle. All other descriptions of known species

describe and show the position of the nerve ring similarly; this fact alone would indicate that this organ is a portion of the oesophagus and not a pharynx. The posterior portion is distinctly more granular and may readily be regarded as the glandular portion of the oesophagus; in fact, both portions more nearly answer the description of the oesophagi of the *Physaloptera* (5).

Pending further histological studies it is proposed that the "pharynx" of *Tetrameridae* be recognized as the muscular oesophagus and the rest of the organ as the glandular oesophagus.

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A ROOT ROT OF SWEET CLOVER AND RELATED CROPS CAUSED BY *PLENODOMUS MELILOTI* DEARNESS AND SANFORD¹

BY G. B. SANFORD²

Abstract

The occurrence, hosts and symptoms of a hitherto undescribed root rot of *Melilotus*, *Medicago* and *Trifolium*, and the relation of temperature and the reaction of substrate to growth of the pathogen *Plenodomus meliloti* are discussed, and its pathogenicity demonstrated. It is suggested that the disease be called "brown root rot".

Evidence is that the hosts mentioned are susceptible only during the winter and early spring dormancy stage. Normal roots of sweet clover, when frozen at -4°C . for four days and subsequently kept at $2-3^{\circ}$, 9° and 16°C ., did not become susceptible. The brown root-rot disease is distinct from true winter injury resulting from insufficient hardiness to cold.

The temperature range for vegetative growth and pycnidia of *P. meliloti* is from 0° to 27°C ., with optimum between 15° and 17°C . Increasingly good growth occurs from 2°C . to optimum temperature. Severe lesions are produced at $2-3^{\circ}$, 9° and 16°C . The optimum pH value for growth in potato dextrose decoction is about 6.2, the other limits being approximately pH 3.2 and 8.2. Soils with an alkaline reaction apparently are unfavorable.

The disease is characterized by brown lesions, on or within which are an abundance of black to dark brown pycnidia. These bodies, 0.5 to 2 mm. in longest diameter, may have one or more spore bearing chambers. Each chamber may have one to several ostioles, through which the one-celled spores, averaging $5.2 \times 2.84 \mu$, exude. The hyphae do not bear spores.

Dissemination of the pathogen by seed would not seem to be of practical importance. Control by crop sanitation is recommended, at least until varieties more resistant are available.

When observing types of winter injury of *Melilotus*, *Medicago* and *Trifolium* in Alberta and Saskatchewan, it occurred to the writer that certain soil-inhabiting micro-organisms might, while the plant was still in the dormant or semidormant condition, seriously increase such injury. As a result of studies begun early in 1926, it is now possible to report, in some detail, a hitherto undescribed root rot of these crops caused by *Plenodomus meliloti* (1). Newton and Brown (5) probably refer to the disease described herein; however, the causal organism was not isolated by them. The writer (7, 8) has also referred briefly to this root rot.

Description of the Disease

The disease is characterized by brown, slightly sunken, necrotic lesions, which appear on the tap or lateral roots and rootlets of sweet clover, alfalfa, and common clover (*Trifolium pratense*). Sometimes the lesions are more common on the roots level with the debris of a former crop. These lesions develop rapidly in all tissues of the host, particularly the pith, as soon as the surface soil thaws in the spring, or even during late winter if the weather is

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mild. Plate I, Fig. 1, illustrates fairly well, the types of lesions which commonly occur on sweet clover roots under field conditions. Any part of the root surface may be affected (Plate I, Fig. 2), although the tips of the roots or smaller rootlets often succumb first. These lesions increase in various degrees, and frequently involve the lower two-thirds or three-quarters of the root system. Sometimes a part of the root system may be left intact (Plate I, Fig. 1, A). Some crown shoots are nearly always produced, even though the tap root is nearly destroyed (Plate I, Fig. 1, C). Often plants, the tap roots of which are severely rotted, but not near enough to the crown to be fatal, survive and partly recover by the aid of new roots produced near the crown. However, such cases usually result in more or less stunted plants. The progress of even severe lesions is terminated by callus when good root development and new growth begin. Since the lesions are dark brown, and, also, usually separated from the sound tissue by a decidedly light to dark brown marginal deposit unlike wound cork (Plate I, Fig. 6), it is suggested that the disease be called "brown root rot".

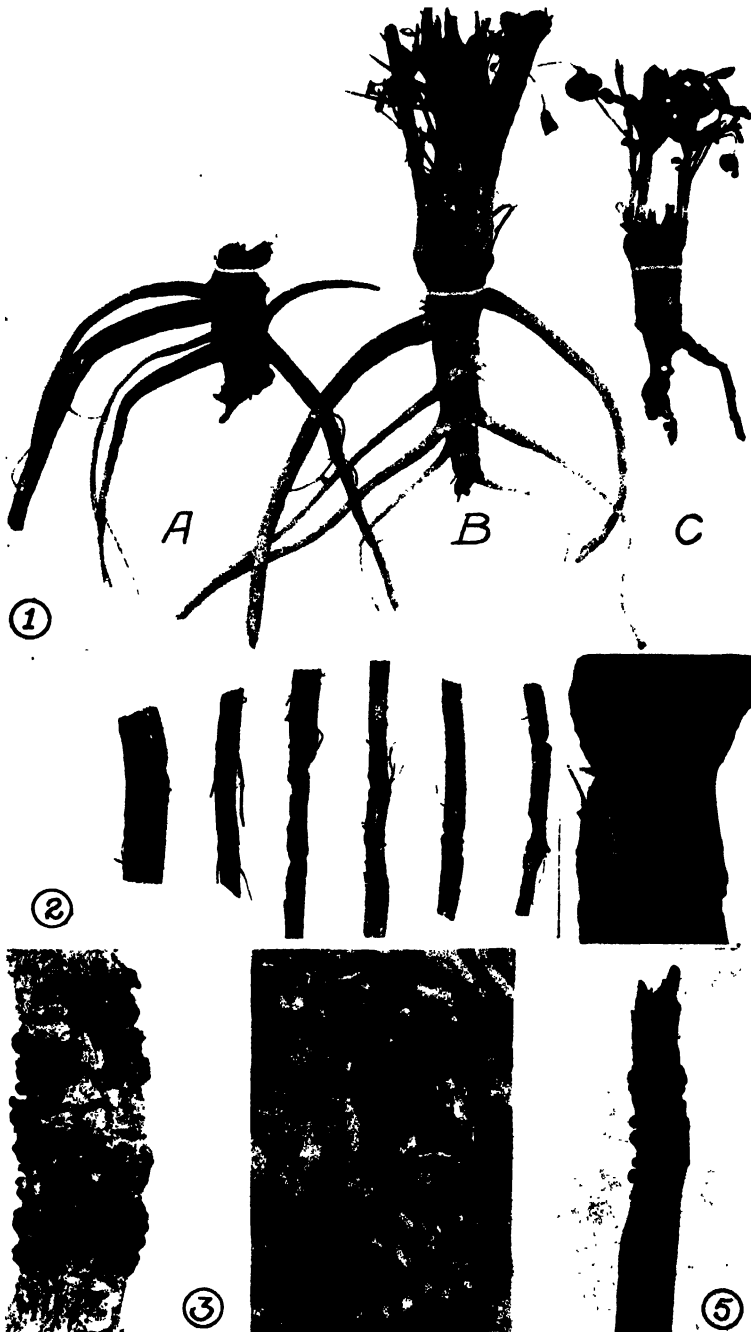
These characteristics rather definitely distinguish the brown root rot from *Sclerotinia* root rot, the lesions of which are softer and typically lack color. Usually pycnidia are very abundant on the surface (Plate I, Fig. 3) of the lesions of brown root rot, and within the necrotic tissue (Plate I, Fig. 4). These are dark brown to black, very closely clustered, and visible to the unaided eye. Sometimes only a few pycnidia, or even none, are obvious. They appear to be produced more abundantly on the lesions formed early, than on those of later incidence. Pycnidia may also occur on the crown portion above ground of dead host plants, although this is not common (Plate I, Fig. 5).

Distribution

Brown root rot has been observed on sweet clover, alfalfa, and common clover growing in the southern and central parts of Alberta and Saskatchewan, as far north as Prince Albert in the latter province, and Athabasca and Beaverlodge in Alberta. The fungus is native and apparently abundant in the cultivated black soils of the prairie area mentioned. The disease has not been reported as occurring elsewhere in Canada, or in foreign countries.

Economic Importance

In the spring of 1926, when the writer began to study this disease, it was prevalent in hundreds of acres of sweet clover which had been "winter-killed", more or less completely, in the area mentioned. In many stands, practically all the plants, regardless of their size, were severely lesioned, and bore an abundance of pycnidia of the fungus associated with brown root rot. The severity of the disease varies greatly from season to season, apparently being influenced by the environment as well as by the physiology of the host, but this is not well understood at present.



FIGS. 1 TO 6. Types of lesions and the location of pycnidia associated with brown root rot on *Melilotus*, under field conditions. 1. A, the tap root rotted first; B, typical lesions on tap and lateral roots (this plant would recover); C, weak, ephemeral crown-shoots on root practically dead. 2. Various types of penetration. 3. Pycnidia characteristically clustered on surface of a lesion. 4. Pycnidia within root tissue. 5. Pycnidia on crown of dead root. 6. The dark brown marginal layer which usually separates the lesion from adjacent sound tissue.

General Description of the Fungus

P. meliloti may be isolated easily from the pycnidia, from lesions next to the sound tissue, or from spores which are produced in the pycnidia. It grows well on a wide range of solid or liquid media, including ordinary potato dextrose agar, sterilized ground oat hulls, corn meal, rice, sterilized potato plugs, and sterilized roots of the host plants. Like many other fungi, the size, shape and color of the mycelium varies greatly according to the kind of substrate, and other factors, such as age, extremes of acidity, or the by-products of metabolism of associated fungi and bacteria. On ordinary potato dextrose and other carbohydrate media, the color of the mycelium, in mass, is light gray to ashen. Microscopically, young hyphae are hyaline and closely septate, but, aged or massed, they become brown with thicker walls. The width of the mycelium varies from 2 to 5 μ , and the cross walls are from 10 to 14 μ apart.

Dark, coriaceous pycnidia are produced abundantly in ordinary light, or in darkness, on a wide range of artificial media, and on the host. They are characteristically superficial on solid media (Plate II, Fig. 1), and on the necrotic tissues of the host plant (Plate I, Fig. 3). They also occur within the tissues (Plate I, Fig. 4) of the host, or develop submerged in solid or liquid media. On the host they are usually regularly subspherical and single, but on artificial media frequently confluent. In longest diameter they range from 0.5 to 2 mm. The initiation of the pycnidia follows closely the growth of the mycelium.

Spore-bearing chambers begin to form when the pycnidium is about 15 days old. As a rule these are initiated about six cells below the upper surface. Ordinarily, spores are produced when the pycnidium is about 50 days old, and these escape through the ostioles from 30 to 50 days later. However, the initiation of the spores may be hastened considerably, and the time for their escape from the pycnidium reduced to 60 days, by incubating pycnidia, 35 days old, in Knop's solution. The spores are hyaline, one-celled, and average 2.8 by 5.2 μ . A pycnidium may have one to several chambers, and as many as 12 or more ostioles. A single chamber may have one to several ostioles, through which the spores escape as a yellow exudate (Plate II, Fig. 1). The morphology of this species will be described more fully in a separate paper.

The Relation of Temperature to Growth

To determine the relation of temperature to growth of *P. meliloti*, the fungus was grown in Petri plates on ordinary potato dextrose agar. Ten of these cultures were incubated at each of the following temperatures: 0-1°, 2-3°, 7-8°, 12°, 15°, 18°, 21°, 23°, 25.5°, and 27° C. The diameters of the colonies on the plates, growing from a small, uniform disk of inoculum, placed at the centre of each plate, were recorded at intervals up to 10 days. The results are indicated by the curve in Text-fig. 1, the cardinal temperatures being 0°, 15-16°, and 27° C. The temperature relation to growth of *P. meliloti*

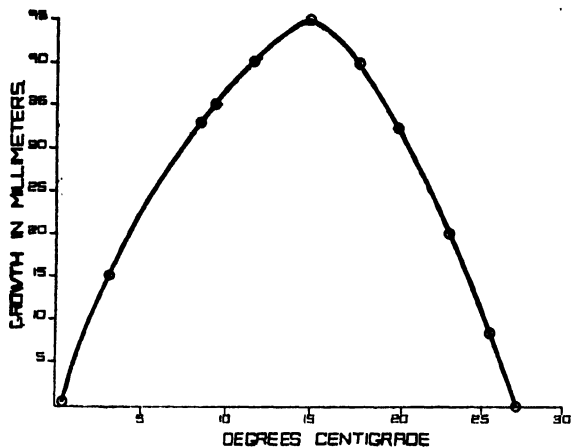


FIG. 1. The curve indicates the growth of *Plenodomus meliloti* on potato dextrose agar at temperatures from 0° to 27° C.

germination of the spores, but no further growth, occurred at 27° C.

furnishes at least one striking contrast to *P. destruens* Harter, the optimum of which Harter (2) found to lie between 21.9° and 30.2° C., the minimum, close to 12.6° C., and the maximum at about 37.3° C.

Pycnospores, fresh from the pycnidium, when placed on microscope slides containing a thin covering of potato dextrose agar, and held at 15° C., germinated abundantly in 48 hr. At 1° and 4° C. superficial growth appeared from spores in ten and seven days, respectively, and at 10° in four days. Slight germination of the spores, but no further growth, occurred at 27° C.

The Relation of the pH Value of the Substrate to Growth

The substrate for this study was the standard potato dextrose decoction, strained, and adjusted to different initial pH values by adding *N* hydrochloric acid to the acid series, and *N*/2 sodium hydroxide to the alkaline series. Each Erlenmeyer flask, containing 100 cc. of the decoction, prepared as mentioned, was inoculated with a loop of spore suspension of *P. meliloti*, and incubated at 15° to 17° C. for 25 days. The pH values of the media in the various series were determined electrometrically at the outset, and every five days thereafter. On the 15th, 20th and 25th days, the dry weight of the fungus was determined, in addition to the pH values of the decoction. The growth of the fungus was ascertained by weighing the dried fungus-mat. The weights and pH values given are the average results from four flasks. These are listed in Table I, and depicted in Text-fig. 2.

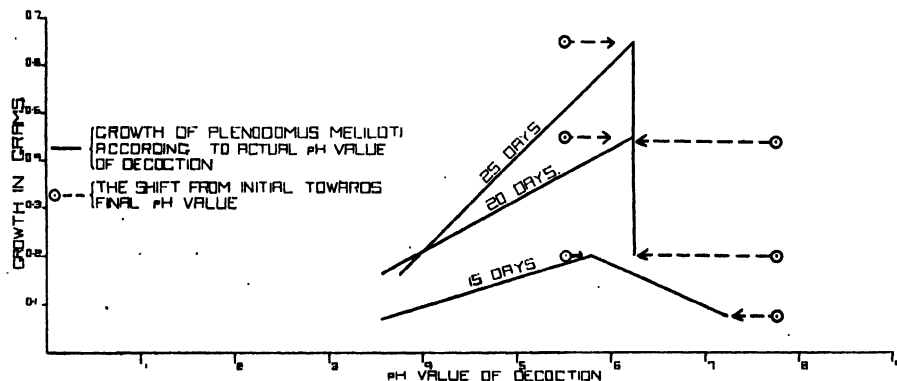
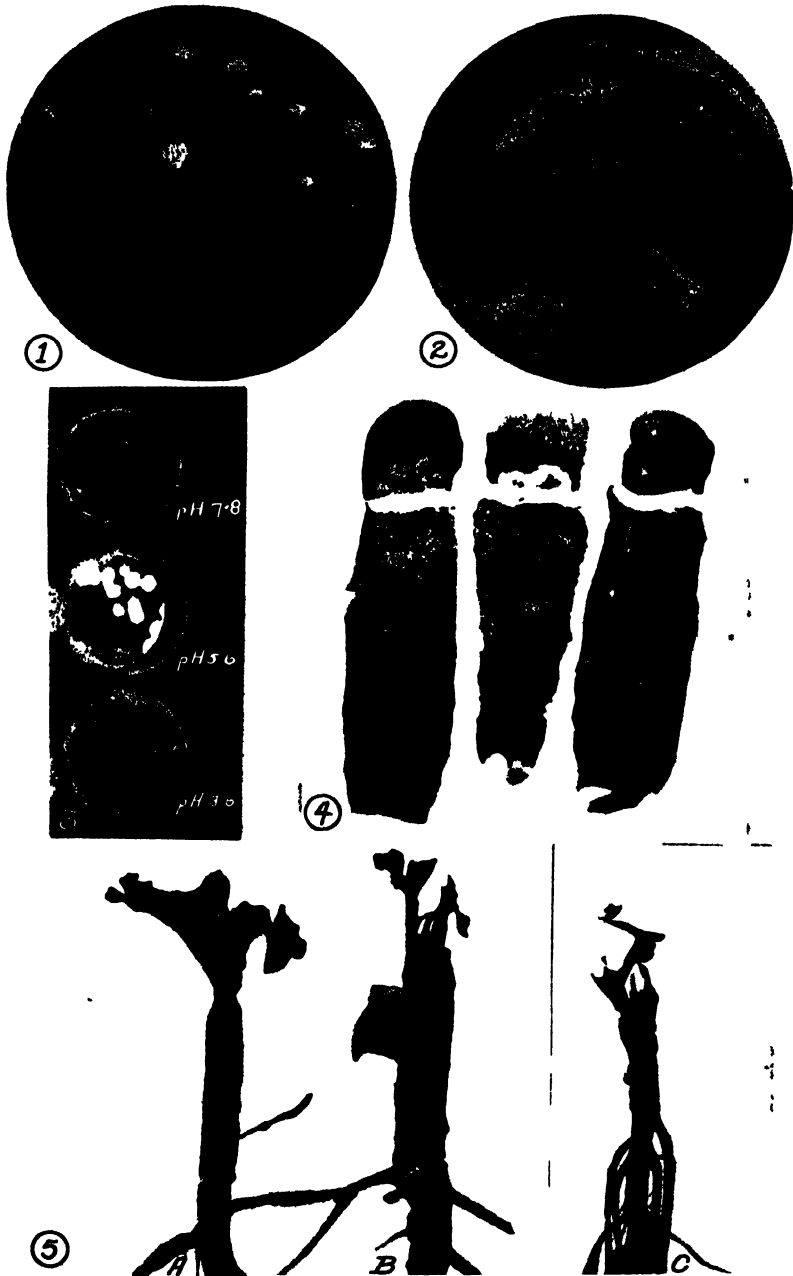


FIG. 2. The curve indicates the growth of *Plenodomus meliloti* in potato dextrose decoction at different initial pH values and temperature 15° to 17° C.



FIGS 1 TO 5. Views of pycnidia and vegetative growth of *P. meliloti* and of brown root rot experimentally produced on roots of *Melilotus* and *Medicago*. 1 Vegetative growth and pycnidia, on nutrient agar, showing ostioles and spore exudate. 2 A section through a pycnidium showing spore bearing chamber. 3 The high per cent germination of spores but restricted growth of colonies, in potato dextrose decoction at pH 3.6, compared to lower per cent germination but freer growth of colonies at pH 7.8. 4 Vegetative growth and pycnidia, which developed in pure culture, on nutrient agar, buried in the soil, during winter and early spring. 5. Brown root rot experimentally produced by placing inoculum against the sound surface of A and B, a lateral and tap root, respectively, of *Melilotus*, and C, a tap root of *Medicago*.

TABLE I

GERMINATION AND SUBSEQUENT GROWTH OF *Plenodomus meliloti* IN POTATO DEXTROSE DECOCTION AT VARIOUS pH VALUES

Media series	Initial	5 Days	10 Days	15 Days		20 Days		25 Days		Remarks
	pH	pH	pH	pH	gm.	pH	gm.	pH	gm.	
I	1.6	1.6	1.6	1.6	.0	1.8	.0	1.8	.0	No growth
II	3.6	3.6	3.6	3.6	.073	3.6	.152	3.7	.152	Delayed germination, slow growth
III	5.6	5.6	5.7	5.8	.221	6.2	.448	6.2	.678	Quick germination, excellent growth
IV	7.8	7.7	7.7	7.2	.077	6.2	.211	6.2	.445	Delayed germination
V	8.8	8.3	8.3	8.3	.0	8.4	.0	8.2	Trace	Few spores beginning growth
Control	5.6			5.5		5.7		5.7		
	4.2	4.2	4.2	4.2		4.2		4.1		Standard buffer solution

The limits for germination and growth were found to be pH 3.2, and pH 8.2, up to 15 days. The series which had initial pH values of 3.6 and 7.8 were about equally favorable up to 15 days, but, subsequently, the advantage was distinctly with the latter series. The spores germinated quickly at initial pH 5.6. At pH 3.6 and 7.8 this was slower, while a considerably higher percentage of the spores germinated at pH 3.6 than at initial pH 7.8, during the first seven days. These results agree with those of Webb (10), *viz.*, that increasing acidity favorably influences the germination of spores. The photograph in Plate II, Fig. 3, taken on the eighth day, illustrates the relative germination of the spores at pH 3.6 and 7.8. The original colonies at pH 5.6 were obscured by general growth within the decoction at this time.

Since after 20 days the pH value of media series IV was raised from 7.8 to 6.2, and the pH value of media series III was depressed from 5.6 to 6.2, it may be assumed that one growth optimum exists near a pH of 6.2. Moreover, the isoelectric point of the living fungus is between a pH 5.5 and 6.0. Referring to the work of Scott (9), another optimum for growth possibly exists between the isoelectric point and the higher pH value of 3.7, but this was not shown, because of the absence of series in this region.

If the growth of *P. meliloti*, as indicated in Table I and Text-fig. 2, is a fair indication of its growth in soils of corresponding pH values, it would appear that the cultivated horizon of typically black or wooded soils of Alberta and Saskatchewan, being commonly between pH 6 and 7, would be favorable to good growth of this fungus. Similarly, the brown soils, typical of the prairie belt, because of their alkaline reaction, commonly being from pH 7.0 to 8 or greater, would be only from slightly favorable to distinctly unfavorable. This observation is based on the work of Wyatt and Newton (12), who state that the pH values of the A₁ horizon of typically wooded soil, black soil, and brown soil, of Alberta, are 6.2, 6.0 and 7.2 to 8 or greater, respectively. Obviously, certain variations in reaction would occur within

each soil belt, according to soil. Data supplied by Mitchell (4), and by Saskatchewan Soil Survey Reports 1 to 9, indicate that, in general, the reaction of corresponding types of soil in that province is similar to those of Alberta.

Pathogenicity

Results of studies on the pathogenicity of *P. meliloti* are given in the following experiments:—

Experiment 1

During March, 1927, sweet clover plants were grown from disinfected seed, in sterilized soil, heavily inoculated with a vigorous culture of the fungus. The soil was kept at 12 to 13°, 17 to 18°, 20 to 21°, and 26 to 27° C., by the aid of temperature control tanks. There were 160 mature plants at each temperature, 40 of which were controls. When these plants were harvested five months later, the roots were apparently healthy. These results suggested that the fungus was not parasitic on normal plants at a temperature favorable to the growth of both plant and pathogen.

Experiment 2

The object here was to determine whether, at high or low soil temperatures, the cutting off of the tap roots of seedling sweet clover plants increases their susceptibility to brown root rot. The soil temperatures were 4 to 5° and 17 to 20° C. The tap roots of the seedling plants were from 2 to 3 mm. in diameter. The tap roots were cut off about two inches below the crowns. Sixty plants, the tap roots of which were cut off, were planted in the inoculated soil at each temperature. The control series for each temperature contained 20 normal plants, as well as 20 plants with tap roots cut off, all planted in sterilized soil.

Seventeen days later the roots were examined. In the inoculated soil, kept at 4° to 5° C., all the roots, whether cut or uncut, were unhealthy, with pycnidia of *P. meliloti* thereon. Likewise, the roots of the control series had many dead areas, and were shrunken and apparently nearly dead, but without pycnidia. The roots of all plants at 17 to 20° C. were growing well and were apparently very healthy.

Experiment 3

This was a repetition of Experiment 2, except that the tap roots were not cut off, and that the low soil temperature was approximately three degrees higher, viz., 5 to 8° C. Vigorous plants, from the same source as those used for Experiment 2, were transplanted into the inoculated and uninoculated soils, at the conclusion of Experiment 2, that is, 17 days after that experiment was begun.

The roots in both temperature series were examined at the end of 48 days. A total of 108 dead areas developed on the roots of the 60 plants in the inoculated soil, while 29 similar areas appeared on the 30 control plants in the uninoculated soil. Very few new roots started to develop at this temper-

ature, and those which the plants had, when transplanted, lost their vigor. At 17 to 20° C. all of the plants grew well, the roots being perfectly healthy.

The results of the foregoing experiments indicated that unfrozen roots of sweet clover, up to four months of age, were immune to *P. meliloti* at soil temperatures 12 to 27° C. Whether such roots were also immune at a temperature range of 4 to 8° C. could not be determined from the experiments made, since the roots of the plants used failed to produce new roots, and also tended to become senile at temperatures from 4 to 8° C.

Experiment 4

The object here was to determine whether first-year roots of sweet clover and alfalfa, growing in the field, were immune to *P. meliloti* prior to winter dormancy. Seed of *Melilotus alba* (varieties—Arctic, Common White and Zouave), and of *Medicago sativa* (varieties—Grimm and Baltic), known to be susceptible, was sown June 15. The roots of the plants were inoculated as they grew in the field August 7. Each test comprised ten roots, inoculated, and an equal number of control roots, located alternately in the row with the inoculated ones. Both test and control roots were treated similarly, except that the latter did not receive inoculum. The fungus was applied to the tissues in three ways: (a), in a cavity made by passing a small cork-borer through the tap root, about two inches below the crown; (b), against the uninjured tap root, about two inches below the crown; and (c), against the cut off ends, and also against the normal ends of lateral roots. In all cases the root surface to be treated was washed with mercuric chloride solution, followed by sterilized water. The inoculum, grown on ground oat hulls, was placed in position and protected by a wrapping of cotton. Finally, the excavated soil was returned about the roots and firmed.

When the roots were removed from the freezing soil at the beginning of November, the treated and control roots, alike, were sound. The cavities which had been made were calloused, and the tap roots had grown well, being from 15 to 20 mm. in diameter. It was concluded, from this and previous experiments, that first-year roots of sweet clover and alfalfa are not susceptible to *P. meliloti* before winter dormancy.

Experiment 5

This was to determine the susceptibility of roots of sweet clover, alfalfa, and common clover to *P. meliloti* during the winter dormancy period. Five varieties of sweet clover, four of alfalfa, and nine of common clover were tested, all of which are named later in the section of this paper entitled "hosts". The three methods of applying inoculum to the roots, mentioned in the foregoing experiment, were used on each of the varieties employed in this test. The use and arrangement of control roots, the preparation of roots for inoculation, and subsequent care, were the same as described in Experiment 4. The roots were treated as the ground froze during early November, and they were examined early next May.

The results of this experiment, with regard to sweet clover and alfalfa, were so definite, and for each so uniform, that they may be given briefly. Every inoculation, regardless of method used, or variety, was successful in producing the lesion characteristic of brown root rot. In general, where the inoculum had been placed in cavities in the tap root, the lesions were more severe than where it had been placed against the uninjured tap root, although in many cases of the latter, maximum injury occurred. The inoculations on the cut or uncut lateral roots were equally effective (Plate II, Fig. 5, *A*), and, in many cases the decay entered the tap root. In Plate II, Fig. 5, *B* and *C*, are photographs of typical lesions of brown root rot, experimentally produced by contact inoculation on sweet clover and alfalfa, respectively.

To indicate the growth of *P. meliloti* during the winter, several slants of potato dextrose agar were inoculated and immediately placed about 4 in. deep in the frozen soil, near the roots. The photograph in Plate II, Fig. 4, illustrates the excellent vegetative growth and abundance of pycnidia, which developed from November until April 15, following.

The roots of common clover also proved to be susceptible to *P. meliloti*, but the degree of this was much obscured by severe winter injury which many of the varieties suffered. However, typical lesions, with pycnidia, were produced experimentally on the two hardier varieties, Late Swedish Red, and Altaswede. On all inoculated roots of other varieties, dead or suffering from ordinary winter injury, there were numerous pycnidia of *P. meliloti*. Probably this fungus was associated chiefly as a saprophyte in the latter instances, but there is still the possibility that it had increased the injury primarily arising from lack of winter hardiness. This phase of the problem is being investigated.

Experiment 6

Since *P. meliloti* penetrated easily and rotted the roots of sweet clover emerging from the dormancy stage (Experiment 5), it was important to determine the effect of soil temperature on the development of the disease. Accordingly, large plants of *M. alba*, from seed planted the previous June, were removed from the frozen field soil March 1. The surface of these roots was treated with mercuric chloride solution, then washed, and inoculated with the pathogen, and planted immediately in sterilized soil of optimum moisture. The inoculum was placed in a cavity in the tap root, as described earlier, and protected by a wrapping of sterilized cotton. The controls were prepared similarly, but without the pathogen. Series, consisting of 30 inoculated roots, and 10 control roots, were kept at 2–3°, 9° and 16° C., respectively. The roots were examined for disease at the end of 21 days.

At 2–3° C., 1 root had a trace of rot; 4 roots were decayed slightly; 8 had light lesions; 12, medium decay, and 4 roots had severe lesions. At 9° C., 3 roots had slight lesions; 5 had light lesions; 13, medium, and 9 roots had severe lesions. Of the roots at 16° C., 2 had slight lesions; 7 light, 12 medium, and 9 severe lesions. The controls were sound. The growth of the crown foliage

on the roots at 16° was good, that at 9° fair, while only slight growth occurred at 2–3° C. If the roots at the beginning of the experiment were about equally susceptible, the development of the disease would depend partly on the growth of the fungus, and partly on the time taken for recovery of the host from dormancy, both of which would be favored by a temperature of 16° C. At 16° and 9° C. the disease developed about equally well, while at 2–3° C. it was retarded. However, if the test had run longer than 21 days the final results might have been reversed.

Experiment 7

This was to determine the progress of brown root rot at temperatures 2–3°, 9° and 16° C. in normal roots of sweet clover, artificially frozen and not frozen. The roots used were from sweet clover plants about three months old, and grown in the greenhouse. These were inoculated by the core method, described in Experiment 5, and planted in sterilized soil of optimum moisture. There were 20 inoculated roots, and 10 control roots in each frozen and unfrozen series of the temperatures mentioned. The roots for the frozen series were first hardened off at 5°, 0°, and –4°, then to 3° C., each change being for four days. The plants of each of the two series mentioned were held at 2–3°, 9°, and about 16° C., respectively, for 21 days, when they were examined for disease.

The results of this experiment were that both inoculated and control roots remained sound at all temperatures, in both frozen and unfrozen series. New root growth at 3° C. on both series was negligible. There was some evidence of senility of the older rootlets. At 9°, new roots developed fairly well, but slowly, while at 16° C. new roots were abundant. Excellent top growth occurred at 16°, it was fair at 9°, and very slow at 2–3° C. Thus normal, unfrozen roots of sweet clover appear to be immune to *P. meliloti* at temperatures above 2° C. Also, the freezing of them at –4° C. for four days does not make them susceptible.

Hosts

Although field tests of the relative resistance of varieties of alfalfa, common clover and sweet clover to *P. meliloti* are still incomplete, experimental evidence to date indicates that sweet clover is more susceptible than either common clover or alfalfa. The following varieties of sweet clover, alfalfa, and common clover were found to be susceptible by tests made in the field: *Sweet Clover*,—Arctic, Common White, Maccor, Zouave, Grundy; *Alfalfa*,—Grimm, Baltic, Cherno, Cossack; *Common Clover*,—Late Swedish Red, Altaswede, St. Clett, Trystofte, Dauphene, Mammoth, Albert, Kenora, Spadone.

Robertson (6) found that a culture of *P. meliloti*, isolated from hollyhock (*Althaea rosea*), was pathogenic to roots of this plant, and also to roots of sweet clover, during winter dormancy.

In addition to the hosts mentioned, pycnidia were found during May on dead roots of *Axyris amaranthoides*, *Amaranthus retroflexus* and *Avena sativa*,

the relationship being strictly saprophytic. Pycnidia were abundant on the first host, very scarce on the second, and only one body was found on oats. Also, no bodies were found on the roots of dead wheat stubble, whereas, on sweet clover, in the same plot, pycnidia were very abundant.

Persistence in Dry Soils

To determine how long this pathogen might survive in dry soils, a loosely plugged 200-cc. flask of sterilized soil was inoculated with a spore suspension of *P. meliloti*, and incubated at about 18° C. for 10 days, until a substantial growth of mycelium had ramified the soil. The culture was then kept at room temperature for two years, during which time fragments of the soil were cultured in Petri plates on potato dextrose agar. The moisture content of the soil at the end of 60 days was 9.3% of its moisture-holding capacity, and at the end of 20 months, 4.4%. The fungus grew from the dry soil up to 20 months, but not longer. Microscopic examination of the soil showed that the fungus persisted in the form of small sclerotia-like masses, which probably were immature pycnidia, since *P. meliloti* has not been observed to produce real sclerotia, either in culture or in the tissue of the host. These results suggest that pycnidia might remain viable in unusually dry soils, at least one year.

Occurrence in Virgin Soils

As *P. meliloti* was so common in recently virgin soils, it seemed probable it was native to strictly virgin prairie sod. Accordingly, the crown portion was removed from 50 vigorous tap roots of *M. alba*, to prevent growth, and the roots disinfected and washed. These were distributed late in October in the black loam virgin prairie sod at Edmonton, and left buried until May, the following spring, at which time the roots were more or less decayed. Several of these roots bore pycnidia from which *P. meliloti* was isolated.

Control

The danger of contaminating new soil, in an area already infested, by means of spores of the pathogen carried by sweet clover seed or foliage, would seem unimportant in practice. Probably the wind is the most effective agent in carrying this fungus from one field to another. Control of brown root rot by crop rotation, or by a naturally resistant variety, seem the only methods available. However, the writer's studies in connection with either method have not yet yielded definite information. Seeding sweet clover in soil where brown root rot has been prevalent would be unsafe, unless preceded by immune crops.

From observation and experiment, brown root rot appears to progress as well in large plants as in small ones. On the other hand, the large roots seem to survive more often than small roots, because small tap roots, with a limited lateral system, are the more easily killed. Thus, large, vigorous plants should be produced the first season, and seeding without a nurse crop is, perhaps, the best way to do this.

Discussion

The foregoing results show that, under field or laboratory conditions, the roots of sweet clover, common clover and alfalfa are not susceptible to *P. meliloti* prior to the winter dormancy stage, but that they become very susceptible during late winter and early spring. The changed biological condition of the internal tissue of the roots, resulting from the long dormant condition, seems to offer the best explanation for this. Whether the marked susceptibility can be attributed to an increase of food materials favorable to the fungus at this time, or to the failure of the cells of the partially dormant tissue to function normally in resisting penetration, are interesting questions. In this connection it was observed that the roots of sweet clover and alfalfa commonly are more or less shrunk by late winter, and that the cells of the root tissue apparently lack turgor. However, when new crown growth starts and new roots are proliferated on the advent of higher soil temperatures, the roots soon return to normal plumpness and rigidity, characteristic of summer growth. Coincident with this return to normalcy is the marked slowing up and final arrest of the disease.

Jones (3) found that factors of climate and soil during winter caused certain internal and external mechanical injuries to the roots of alfalfa. Weimer (11) produced a number of these injuries, including the killing of the tender root tips, by artificial freezing. Macroscopic evidence during these studies indicated that similar external injuries occur on the root tips of sweet clover during winter in western Canada, and that such areas greatly favor the incidence of brown root rot. On the other hand, there were numerous cases where *P. meliloti* entered the root surface in the absence of rootlets or mechanical injuries.

With regard to the effect of low soil temperatures on brown root rot of sweet clover, while in a dormant or semidormant condition, evidence during the last four years has been that inoculated roots, at Edmonton and Lacombe, have suffered as severely in one year as in another, while uninoculated plants have remained sound. Thus, true winter-killing and brown root rot are distinct.

Finally, if climatic and other factors, during winter and early spring, alter the complete resistance of the roots to a condition of marked susceptibility to *P. meliloti*, various degrees of this might be expected among varieties and roots within a variety, depending upon the reaction of their individual physiology to the environment. Similarly, in the case of true winter-killing, there would be various degrees of physiologic modification. Therefore, it seems probable that other soil-inhabiting fungi and bacteria, under certain conditions, contribute to the mortality arising from lack of winter hardiness, as does *P. meliloti*.

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THE RELATION OF SPECIES OF AGROPYRON AND CERTAIN OTHER GRASSES TO THE FOOT-ROT PROBLEM OF WHEAT IN ALBERTA¹

By G. W. PADWICK² AND A. W. HENRY³

Abstract

A survey was made of certain wild and cultivated grasses in Alberta in order to ascertain to what extent they are harboring fungi capable of causing foot rots of wheat. Quack grass, *Agropyron repens*, and western rye grass, *Agropyron tenerum*, were found to be particularly important in this respect. Both were found heavily attacked under natural conditions with strains of the take-all fungus, *Ophiobolus graminis*, which proved capable of causing as severe damage to wheat as strains from wheat. Strains of *Helminthosporium sativum* which proved highly pathogenic to wheat were also isolated from these two grasses. Strains of *Fusarium* obtained from *A. repens* and *A. richardsonii* caused little or no damage to wheat.

Agropyron repens is already an important weed in central Alberta. It was found infesting cultivated fields in summerfallow as well as those in crop. In summerfallow this weed appears to encourage the survival of *Ophiobolus graminis*, while in wheat fields infected quack grass was found associated with severe take-all damage to the crop. *Agropyron tenerum* is one of our most popular forage grasses and occurs commonly as a wild native plant in Alberta. Observations indicate that in the moister parts of the province wheat following this grass in rotations may be severely injured by take-all. In a rotation at the University of Alberta, wheat showed little or no take-all damage after timothy and alfalfa, moderate damage after brome grass and severe damage after western rye grass. In this experiment western rye grass itself was almost killed out prematurely in all replicates, apparently by the take-all fungus.

Artificial inoculation of the various grasses was made with wheat strains of foot-rotting fungi by adding inoculum to the soil. All species of *Agropyron* tested including crested wheat grass, *Agropyron cristatum*, proved highly susceptible to *Ophiobolus graminis*, moderately susceptible to *Helminthosporium sativum*, but only slightly susceptible to *Fusarium graminearum*, though the latter was responsible for considerable non-emergence of the seedlings. *Bromus inermis* and *B. ciliatus* proved quite susceptible to all three pathogens. *Hordeum jubatum* was heavily attacked by *Ophiobolus graminis* but not by the other two fungi. *Avena sativa* was not attacked by *O. graminis* and only slightly by *H. sativum* and *F. graminearum*, while timothy, *Phleum pratense*, appeared immune from all three fungi.

Introduction

Numerous reports have been made of fungi causing foot rots of wheat on wild and cultivated grasses including members of the genus *Agropyron*. The purpose of the present investigation was to determine as far as possible the extent to which some of these grasses serve in increasing the damage to wheat caused by foot rots and to find whether the various grasses common in Alberta exhibit any appreciable differences in the extent to which they intensify the problem.

Early workers reporting the production of perithecia of *Ophiobolus graminis*, the organism causing take-all of wheat, on species of *Agropyron* were Saccardo in 1875, Waters (13) in 1920, and Brittlebank (1) in 1919. Waters reported production of perithecia on *A. repens* and Brittlebank on *A. scabra*.

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Kirby (5) in 1921 found *A. repens* commonly affected with take-all in New York State under natural conditions. In addition it was found that all six species of *Agropyron* studied, namely, *A. caninum*, *A. cristatum*, *A. intermedium*, *A. repens*, *A. smithii* and *A. tenerum* became infected when seeded in pots of clean unsterilized soil which had inoculum added in the form of pure fungous mycelium grown on steamed wheat kernels. Perithecia were produced by all six species in varying numbers, the writer stating that, "since the important role of the grasses would be as carriers of the disease the chief object of this test was the determination of the grasses on which perithecia were produced". The same writer (6) in 1925 conducted a still more extensive experiment, this time using sterilized soil. Together with many other grasses, 11 species of *Agropyron* were tested, namely, *A. caninum*, *A. cristatum*, *A. desertorum*, *A. intermedium*, *A. obtusiusculum*, *A. repens*, *A. richardsonii*, *A. scabrum*, *A. smithii*, *A. spicatum* and *A. tenerum*, on all of which perithecia were developed. The only occurrence in the field, however, was in the case of *A. repens*. The writer again states that the importance of any grass as a harbinger of the pathogene causing take-all of wheat depends on the number of perithecia produced.

Russell (11) believes that brome grass (*Bromus inermis*), western rye grass (*A. tenerum*) and quack grass (*A. repens*) all serve to increase the amount of take-all in crops of wheat following them in the rotation.

Bolley in 1911 found a species of *Helminthosporium* causing infection of *A. repens* in North Dakota and Wisconsin, and apparently regarded it as identical with that causing foot rot of wheat. Dreschler (4) found *H. sativum* commonly caused a leaf-spot disease of *A. repens* in the midwestern states of the United States. Other investigators report successful inoculation of hosts in several different genera with *H. sativum*. Stakman (12) in 1920 inoculated the leaves of a number of grasses with strains of *H. sativum* obtained from wheat and rye, and successfully infected those of *Agropyron smithii*, *A. repens*, and *Hordeum jubatum*. No leaf lesions appeared on *Phleum pratense*. The root systems were not inoculated. Christensen (2) succeeded in isolating a species of *Helminthosporium* which he believed to be *H. sativum* from above-ground parts of *A. caninum*, *A. desertorum*, *A. repens*, *A. smithii* and *A. tenerum*, as well as from a number of other grasses under natural conditions in various parts of the United States. In pots of sterilized soil in the greenhouse the aboveground parts of seven species of *Agropyron* inoculated with *H. sativum* all showed infection to a more or less marked degree. Heavy infection occurred on *A. caninum*, *A. spicatum* and *A. tenerum longifolium*; *A. tenerum* and *A. repens* showed medium infection, and only light infection was apparent on *A. cristatum*, *A. desertorum* and *A. smithii*. In these cases root infections were not studied especially, but a small additional experiment showed the roots of *A. tenerum* to be infected. Attention is called to several possibilities in relation to these and other susceptible grasses; that they may serve to increase secondary infection of cereals; that they may aid in overwintering; and that windblown spores from foliages of susceptible grasses may cause

infection of wheat on comparatively clean soil. These workers did not especially direct their attention to the importance of these grasses in connection with the foot-rot problem, but instead restricted their studies largely to the leaf-spot diseases.

Several workers have reported the occurrence of *Giberella saubinettii*, the perfect stage of *Fusarium graminearum*, under natural conditions on *Agropyron smithii*, *A. repens*, *A. tenerum* and *A. caninum* causing head blights, and the list was extended by MacInnes and Fogelman (8) at Minnesota in 1923 to include under experimental conditions a number of grasses, amongst them *Agropyron desertorum*, *Bromus inermis* and *Phleum pratense*.

Distribution of Grasses in Alberta

Ample literature is available concerning the distribution of the more important grasses in Alberta. Many of the most important species in relation to the foot-rot problem of wheat occur in the genus *Agropyron*, which has been chosen mainly for this investigation. However, several other genera contain native or introduced species with a wide distribution in Alberta, and the more common of these are included, namely, *Bromus inermis*, *B. ciliatus*, *Hordeum jubatum*, *Avena fatua* and *Phleum pratense*. The distribution of these grasses in Alberta will be described briefly, and for more detailed information the investigations of Clarke, Moss, Peto and Preston may be consulted (3, 7, 9, 10).

Agropyron tenerum and *A. richardsonii*

These grasses are distributed throughout a large part of the province of Alberta, occurring most commonly in the northern and parkland belts. Western rye grass (*Agropyron tenerum*) is widely cultivated in the province.

Agropyron repens

This weed has become common in many parts of Alberta, especially in the central part, its persistence being due largely to its numerous creeping root-stocks. Quack grass is prevalent in the Edmonton district, and during the summer of 1932 the writers observed few summerfallow fields in this district completely free from the weed. In some fields of well-worked fallow where all other weeds were kept well under control, quite thick stands of quack grass were noticeable in patches throughout the fields. At least three fields of almost pure quack grass were noticed which were being used for hay.

Agropyron smithii

This is a common constituent of the short grass plains, occurs to a considerable extent in the transition belt or parkland areas, and is found in central and northern Alberta as a rule only on drier and more exposed slopes. It usually does not encroach seriously upon cultivated fields except in very dry areas.

Agropyron caninum

This species has been reported to occur amongst the upland grasses of the Grande Prairie-Beaverlodge district of northern Alberta. It is not a common grass in central and southern Alberta.

Agropyron griffithsii

A. griffithsii occurs mainly on the dark brown soils of the prairie. It does not as a rule form a thick sod and seems to occur mainly as a few scattered plants within a district. It rarely occurs in cultivated fields.

Agropyron cristatum

This grass (known under the common name of crested wheat grass), was introduced from European Russia, and is recommended for cultivation in districts of limited rainfall. The grass spreads to some extent by underground stems, but does not produce long creeping rhizomes like those found in *A. repens*. The likelihood of more extensive use of this grass as a cultivated orage crop in the future is to be considered.

Agropyron dasystachyum

This grass resembles *A. smithii* in its distribution, being found mainly in the southern part of the province, but is less tolerant towards excessive moisture and is therefore fairly well restricted to the southern and drier parts of the province.

Agropyron sibiricum, *A. obtusiusculum*, *A. desertorum* and *A. elongatum*

For the most part natives of Russia, these grasses have been introduced mainly for experimental purposes. In view of the possible development of some of these species they were studied to some extent in connection with the host range of the foot-rotting organisms concerned.

Bromus inermis

Awnless brome grass is grown to a large extent in Alberta as a hay and pasture crop, especially in central Alberta. It is quite commonly grown on sandy land, but is distributed over all types of soil. Throughout central Alberta it has escaped from cultivation, often growing thickly along roadsides. It is a moderately difficult grass to eradicate.

Bromus ciliatus

This is a native brome grass found commonly in northern and central Alberta but as a rule not forming a thick sod. It is found quite commonly on sandy lands and hillsides. It is not a serious weed.

Hordeum jubatum

H. jubatum is a perennial grass, distributed throughout Alberta, and seems equally suited to dry sandy soils and moist bottom lands. It occurs commonly in cultivated fields and spreads rapidly by seed; hay crops are frequently badly contaminated with this weed. It is prevalent on waste land.

Avena fatua

It is hardly necessary to say more than that this weed is generally distributed throughout Alberta and is very commonly found in cultivated crops.

Phleum pratense

Timothy is cultivated in some of the moister districts, and is found to a small extent escaped from cultivation.

Occurrence of Foot-rotting Organisms of Wheat on Grasses Under Natural Conditions

During the summer of 1932 a brief survey was made of several districts of Alberta with a view to determining the frequency of occurrence of wheat foot-rotting organisms on grasses. This work was done in central Alberta, automobile trips being made from Edmonton, south to Innisfail, east to Provost and Lloydminster on the Saskatchewan border, and north from Edmonton to Clyde and Westlock. Notes made on a few of the fields examined, together with some observations made in the Edmonton district at previous dates, are summarized below according to the species of host.

Agropyron repens

One of the first isolations made was that of *Helminthosporium sativum* from *A. repens* at Edmonton in September, 1931.

At Edmonton in June 1932, a field was examined which had been left uncultivated for several years and had become infested with quack grass, which was found to be stunted in large circular patches. *Ophiobolus graminis* was isolated without difficulty from rhizomes in these patches. The blackened appearance of these rhizomes is well demonstrated in Fig. 1. The strain was numbered 104.

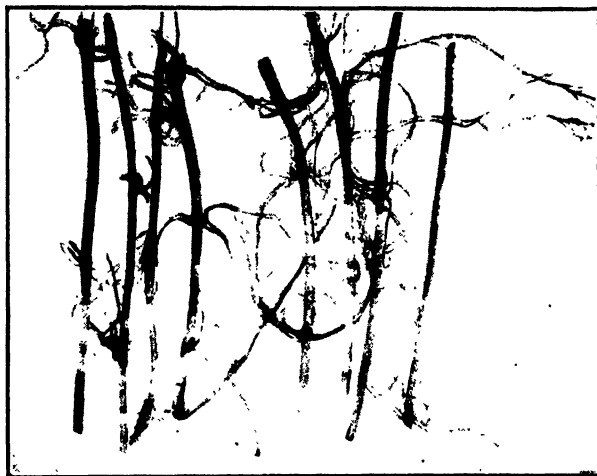


FIG. 1. Left: Rhizomes of *A. repens* from a stunted patch affected with *O. graminis*. Right: Healthy rhizomes from just outside the patch.

A field of *A. repens* of about 10 acres was found at Blackfoot, August 25. The field had been cut for hay and the quack grass was stretching far into adjacent fields of wheat and barley. The rhizomes were heavily affected with take-all. The wheat in the infested field was also severely damaged by take-all, the damage being estimated at 30% and the degree of infection at 51%.

Quack grass was found to be a common weed in a 100-acre field of western rye grass at Vermilion, August 25. There was severe infection of the rhizomes and *Helminthosporium sativum* and a *Colletotrichum* sp. were isolated.

A 20-acre field of stubble was examined at Vegreville on August 25. It had apparently been intended for summerfallow but had not been worked up to this date. Considerable quack grass was present, heavily affected with take-all caused by *O. graminis*.

At Josephburg, August 26, a summerfallow field was noticed in which the distribution of quack grass was general. Numerous lesions appeared on the rhizomes and *O. graminis* was identified as the causal organism.

Heavy infestation of summerfallow with *A. repens* was observed at Fort Saskatchewan, August 26. The rhizomes showed symptoms typical of those caused by *O. graminis*, though the fungus was not isolated.

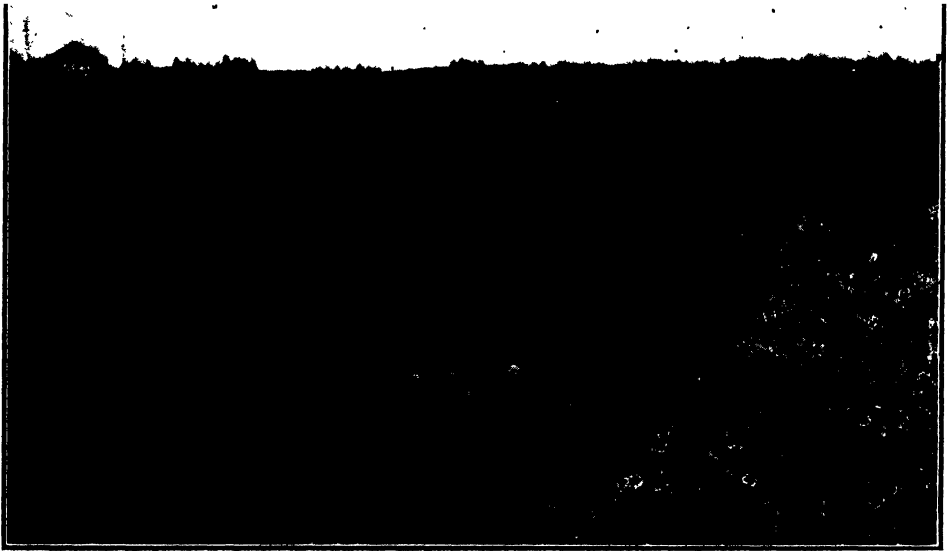


FIG. 2. Right foreground: A western rye grass plot, showing areas where *A. tenerum* plants have been killed and a number of encroaching grasses and weeds. Left foreground: timothy. Right background: alfalfa. Left background: brome.

A summerfallow field of 300 acres examined at Fort Saskatchewan on August 26 was infested with *A. repens* heavily infected with *O. graminis*. A *Fusarium* sp. was also isolated.

A wheat field of about 50 acres at Oliver was found heavily infested with quack grass resulting in pronounced stunting of the wheat. The quack grass was very heavily infected with *O. graminis*, and the wheat showed as much as 10% damage and 30% infection in patches.

Only two cases were found in which the rhizomes of *A. repens* were free from lesions. *O. graminis* was suspected in many cases other than those reported.

Agropyron tenerum

Experimental plots of western rye grass at the University of Alberta were found in May 1932 to be dying out. There is evidence that this was due to a large extent to foot rot. *O. graminis* was isolated from many of the roots. *H. sativum* was also isolated. The history of the plots is described later, but the severe damage and subsequent invasion by weeds can be seen from the photograph (Fig. 2).

A field of about 100 acres at Vermilion on August 25 appeared to be suffering severely from foot rot. *H. sativum* was isolated. The symptoms of take-all appeared to be present, but attempts to isolate *O. graminis* were unsuccessful.

Species of *Fusarium* and an organism resembling a *Leptosphaeria* sp. were isolated in several instances from *A. tenerum*.

Bromus inermis

Foot rot was found in only six out of twenty-three fields of *B. inermis* examined, and in no instance did the damage appear severe. *Helminthosporium sativum* and a *Fusarium* sp. were isolated, but *Ophiobolus graminis* was not found on this grass even when it was growing amongst infected plants of *A. repens*. It is possible, however, that the organism was present on the fine roots of the grass.

Foot rots were also observed on *Agropyron richardsonii* and *A. smithii*, and *H. sativum* was isolated from the former.

In all, only 29% of the fields of *Bromus inermis* examined appeared to be affected with foot rot, while 64.3% of the *Agropyron tenerum* fields and almost all the *A. repens* fields were affected.

Pathogenicity of *Ophiobolus graminis*, *Helminthosporium sativum*, and *Fusarium* spp.

It is of importance to know whether those strains of foot-rotting organisms found on grasses are capable of causing damage to wheat. For this reason several strains of each fungus isolated from grasses were compared with strains of similar organisms obtained from wheat. The tests with *H. sativum* strains were conducted in October 1931; the tests with *O. graminis* and *Fusarium* strains were conducted almost a year later but the methods adopted were similar. The strains used are described below.

Ophiobolus graminis

Strain 103. From wheat grown on soil from plots of *A. tenerum* at the University of Alberta.

Strain 108. Obtained from *A. repens* at Oliver, Alberta.

Strain 104. From rhizomes of *A. repens* near Edmonton, Alberta.

Strain 101. Obtained from *A. tenerum* at the University of Alberta.

Helminthosporium sativum

Strain 6. Obtained from wheat at Brooks, Alberta. This was originally a monospore culture.

Strain 102. From *A. repens* rhizomes at Edmonton, Alberta.

Strain 101. Obtained from the bases of stems of *A. tenerum* on plots at the University of Alberta.

Fusarium Strains

Strain 2. *Fusarium graminearum* from wheat at New Norway, Alberta.

Strain 101. From wheat at Peace River, Alberta.

Strain 102. From *A. repens* at Oliver, Alberta.

Strain 103. Isolated from *A. richardsonii* at Oliver, Alberta.

Soil cultures of the different isolations were grown in Erlenmeyer flasks, eight replicates of each being used in the *H. sativum* tests, but only three replicates of each strain of *Fusarium* and *O. graminis*. For the *Ophiobolus* cultures 10% of cornmeal was added to the soil. To each Erlenmeyer flask 50 gm. of soil (or soil and cornmeal) was added, and 28 cc. of tap water. The flasks were placed in the autoclave and the contents sterilized at 15 lb. pressure for one hour, after which the cultures were started by introducing the fungi. After seventeen days' growth the contents of each flask was added to a pot of sterilized soil, and each pot was seeded with 25 seeds of Marquis wheat and placed in the greenhouse. After nearly three weeks' growth the pots were harvested and the degree of infection and length of the plants were measured. The results are summarized in Tables I, II and III.

TABLE I
PATHOGENICITY OF STRAINS OF *Ophiobolus graminis* ON MARQUIS WHEAT

Strain of organism, and origin	Emergence of wheat plants, %	Average length of plants, cm.	Average degree of infection, %
103 From wheat	82.7	10.1	72.9
108 From <i>A. repens</i>	68.0	8.8	87.4
101 From <i>A. tenerum</i>	80.0	9.9	74.0
104 From <i>A. repens</i>	70.7	8.6	83.0
Check—no organism	76.0	19.3	1.4

TABLE II
PATHOGENICITY OF STRAINS OF *Helminthosporium sativum* ON MARQUIS WHEAT

Strain of organism, and origin	Emergence of wheat plants, %	Average length of plants, cm.	Average degree of infection, %
6 From wheat	47.0	10.7	51.9
101 From <i>A. tenerum</i>	43.0	11.3	41.7
102 From <i>A. repens</i>	57.0	9.4	55.8
Check—no organism	87.5	16.0	1.0

TABLE III
PATHOGENICITY OF STRAINS OF *Fusarium* ON MARQUIS WHEAT

Strain of organism, and origin	Emergence of wheat plants, %	Average length of plants, cm.	Average degree of infection, %
2 From wheat	9.3	6.7	80.0
101 From wheat	61.3	14.9	0.0
102 From <i>A. repens</i>	66.0	14.7	6.0
103 From <i>A. richardsonii</i>	57.3	17.2	0.0
Check—no organism	76.0	19.3	1.4

It is seen that strains of *O. graminis* and *H. sativum* isolated from grasses are generally as pathogenic as are the strains isolated directly from wheat itself. There appear to be slight differences in pathogenicity between the different strains of each of these organisms, and for *Helminthosporium* it was found that these differences were possibly significant in the light of the probable errors, which were calculated but are not included in the tables. These differences were not considered to be of practical importance. The fact that strains of these two organisms obtained from species of *Agropyron* growing under natural conditions are very pathogenic to wheat, however, is of considerable practical significance. The *Fusarium* strains obtained from grasses, with the possible exception of that obtained from *A. repens* were not pathogenic on wheat under the conditions of the experiment, but other experiments (since completed) go to show that these grasses may nevertheless under some conditions harbor forms of *Fusarium* pathogenic towards wheat. The common occurrence of the latter condition in nature is not yet proved.

The Host Range of *Ophiobolus graminis* Sacc., *Helminthosporium sativum* P.K.B. and *Fusarium graminearum* Schwabe

Previous work on the host range of *O. graminis* has been confined mainly to determination of the host species on which the fungus produces perithecia, while with the other two organisms the work has been confined mainly to the aboveground parts of the hosts.

While such methods of determining the reaction of the various grasses may be useful for certain purposes, it seemed that in relation to the foot-rot problem of wheat it was more important to determine the reaction of the basal or underground parts of the grasses to soil-borne inoculum. It was considered that such determinations would also give a better index of the relation which the various grasses might have to the infestation of the soil. Consequently all of the data reported are based on the reaction to soil-borne inoculum, prepared and used as described below.

The strains of fungi used in the study were: *O. graminis*, 108; *H. sativum*, 6; and *F. graminearum*, 2. Litre flasks containing 200 gm. each of soil with 10% cornmeal added were moistened with 112 cc. each of tap water and sterilized.

The organisms were then added, using six flasks

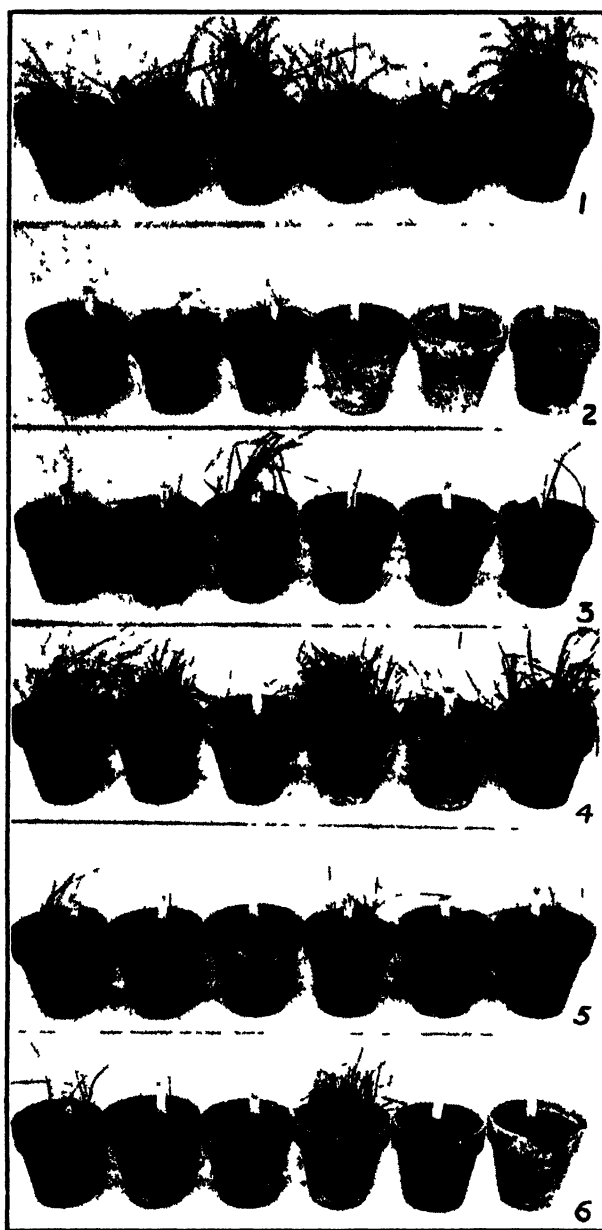


FIG. 3. Effect of soil-borne inoculum of *Helminthosporium sativum* and *Fusarium graminearum* on *Agropyron* spp. Left to right (rows 1-3): *A. cristatum*, *A. sibiricum*, *A. obtusiusculum*, *A. smithii*, *A. desertorum*, *A. caninum*. Row 1, check (no organism added to soil); row 2, soil infested with *H. sativum*; row 3, soil infested with *F. graminearum*. Left to right (rows 4-6): *A. elongatum*, *A. richardsonii*, *A. griffithsii*, *A. tenerum*, *A. dasystachyum*, *A. repens*. Row 4, check (no organism added to soil); row 5, soil infested with *H. sativum*; row 6, soil infested with *F. graminearum*.

for *Fusarium* and twelve each for *Ophiobolus* and *Helminthosporium*. This inoculum was allowed to grow for 17 days at room temperature. Pots of sterilized soil, to which were added separately the three different organisms, were seeded with the various species of grasses. A duplicate series was planted. In addition a similar series was prepared in which unsterilized soil was used. The inoculum was then added as evenly as possible to infest the soil as required and the pots were then seeded to approximately equal numbers of seeds of the various grasses. They were placed in the greenhouse at an air temperature of approximately 70° F. and were grown for 52 days. The pots were photographed (Fig. 3), and the plants harvested, measured and scored for infection. Roots from the *H. sativum* and *F. graminearum* series were surface sterilized with silver nitrate and plated out on potato dextrose agar where infection was suspected; plants and roots of the *Ophiobolus* series were examined with a microscope. The combined results are found in Tables IV and V.

While in this host range study special attention was given to the genus *Agropyron*, several species of grasses occurring as weeds or as crop plants in Alberta and belonging to other genera were included. Outside the genus *Agropyron*, the grasses studied were *Bromus inermis*, *B. ciliatus*, *Hordeum jubatum*, *Phleum pratense* and *Avena fatua*.

Agropyron elongatum was not tested with *O. graminis*. All other tested species of this genus* were susceptible when grown on both sterilized and unsterilized soils. *Bromus inermis*, *B. ciliatus* and *Hordeum jubatum* were all infected, but *Avena fatua* and *Phleum pratense* appeared not to be susceptible (see Fig. 4).

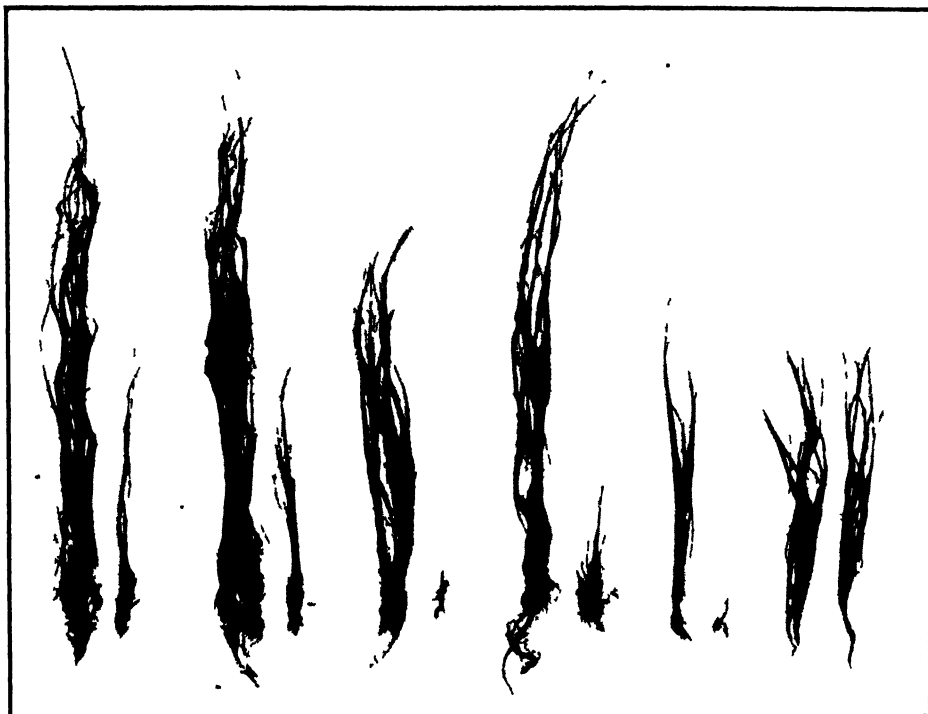


FIG. 4. Effect of *O. graminis* on some grasses of economic importance. Check plants at left and plants from infested soil at right. Left to right: *Agropyron tenerum*, *A. repens*, *A. cristatum*, *Bromus inermis*, *Hordeum jubatum* and *Phleum pratense*.

In the genus *Agropyron* all species were susceptible to *H. sativum*. The only cases where the organism was not isolated were with *A. caninum* on sterilized soil and with several species (*A. cristatum*, *A. desertorum*, *A. elongatum* and *A. griffithsii*) where plants were completely killed and the tissues had become so invaded by saprophytic fungi as to inhibit growth of *H. sativum* when plated on potato dextrose agar. *Bromus inermis* and *B. ciliatus* were both attacked though not severely injured and *Avena fatua* was infected lightly on sterilized soil. *Hordeum jubatum* and *Phleum pratense* were not infected (see Fig. 3).

*The writers are indebted to Dr. J. R. Fryer for supplying seed of these grasses.

TABLE IV
REACTION OF CERTAIN GRASSES TO *O. graminis*, *H. sativum* AND *F. graminearum* IN STERILIZED SO

Organism	<i>Ophiobolus graminis</i>			<i>Helminthosporium sativum</i>			<i>Fusarium graminearum</i>			Check— no organism	
	Av. plant height, cm.	Av. degree of infection, %	Results of isolation trials	Av. plant height, cm.	Av. degree of infection, %	Results of isolation trials	Av. plant height, cm.	Av. degree of infection, %	Results of isolation trials	Av. plant height, cm.	Av. degree of infection, %
<i>A. cristatum</i>	2.0	100.0	+	No plants	19.2	0.0	—	21.1	0.0
<i>A. sibiricum</i>	2.7	100.0	+	22.5	60.0	+	17.8	0.0	—	25.8	0.0
<i>A. obtusiusculum</i>	4.9	100.0	+	16.4	21.8	+	30.7	0.0	—	33.2	0.0
<i>A. smithii</i>	No plants	10.8	40.0	+	15.0	0.0	—	13.8	0.0
<i>A. desertorum</i>	3.5	100.0	+	No plants	No plants	27.7	0.0
<i>A. caninum</i>	7.0	77.6	+	13.8	29.7	—	19.2	0.0	+	21.2	0.0
<i>A. elongatum</i>	Not seeded	21.2	21.4	+	28.6	4.0	—	26.6	0.0
<i>A. richardsonii</i>	9.3	93.2	+	13.4	40.0	+	14.0	0.0	—	15.8	0.0
<i>A. griffithsii</i>	3.8	98.4	+	No plants	No plants	15.1	0.0
<i>A. tenerum</i>	7.1	88.2	+	14.2	23.1	+	19.2	0.0	—	21.5	0.0
<i>A. dasystachyum</i>	5.8	86.7	+	12.7	40.0	+	No plants	18.5	0.0
<i>A. repens</i>	10.4	67.9	+	15.5	36.0	+	24.0	5.0	+	24.2	0.0
<i>Bromus inermis</i>	4.6	95.2	+	18.8	41.3	+	20.5	16.1	+	19.4	0.0
<i>B. ciliatus</i>	No plants	6.9	22.3	+	8.2	6.3	+	7.6	0.0
<i>Hordeum jubatum</i>	6.2	66.7	+	9.7	0.0	—	13.1	0.0	—	15.1	0.0
<i>Avena fatua</i>	33.5	0.0	—	28.7	18.7	+	39.6	0.0	—	36.5	0.0
<i>Phleum pratense</i>	15.2	0.0	—	14.1	0.0	—	19.2	0.0	—	20.3	0.0

TABLE V

REACTION OF CERTAIN GRASSES TO *O. graminis*, *H. sativum* AND *F. graminearum* IN UNSTERILIZED SOIL

Organism	<i>Ophiobolus graminis</i>			<i>Helminthosporium sativum</i>			<i>Fusarium graminearum</i>			Check— no organism	
	Av. plant height, cm.	Av. degree of infection, %	Results of isolation trials	Av. plant height, cm.	Av. degree of infection, %	Results of isolation trials	Av. plant height, cm.	Av. degree of infection, %	Results of isolation trials	Av. plant height, cm.	Av. degree of infection, %
<i>A. cristatum</i>	5 3	80 0	+	No plants			13 2	0 0	—	12 7	0 0
<i>A. sibiricum</i>	7 2	20 0	+	12 8	10 0	+	16 6	0 0	—	14 6	0 0
<i>A. obiusculum</i>	4 3	100 0	+	20 1	20 0	+	17 2	0 0	—	22 5	0 0
<i>A. smithii</i>	No plants			10 0	20 0	+	No plants			12 5	0 0
<i>A. desertorum</i>	2 0	100 0	+	No plants			12 5	0 0	—	16 1	0 0
<i>A. caninum</i>	5 0	89 6	+	12 0	20 0	+	19 0	0 0	—	18 0	0 0
<i>A. elongatum</i>	Not seeded			No plants			20 0	0 0	—	20 7	0 0
<i>A. richardsonii</i>	8 9	89 6	+	12 9	22 4	+	15 7	0 0	—	13 6	0 0
<i>A. griffithsii</i>	5 3	60 0	+	13 6	10 9	+	12 4	0 0	—	11 4	0 0
<i>A. tenerum</i>	5 6	96 0	+	17 2	26 7	+	15 3	0 0	—	14 4	0 0
<i>A. dasystachyum</i>	4 8	93 3	+	8 8	40 0	+	14 4	0 0	—	14 0	0 0
<i>A. repens</i>	8 3	64 6	+	13 9	20 0	+	19 1	0 0	—	16 4	0 0
<i>Bromus inermis</i>	5 3	92 0	+	14 4	33 6	+	13 2	0 0	—	15 7	0 0
<i>B. ciliatus</i>	3 2	100 0	+	6 5	33 3	+	7 7	11 4	+	7 6	0 0
<i>Hordeum jubatum</i>	No plants			10 7	6 4	+	11 5	0 0	+	10 5	0 0
<i>Avena fatua</i>	27 6	0 0	—	26 1	6 0	—	27 6	6 0	+	23 1	0 0
<i>Phleum pratense</i>	10 5	0 0	—	11 7	0 0	—	15 7	0 0	—	11 3	0 0

In many cases a high percentage of the seedlings was killed before emergence by *F. graminearum*, and there were very few cases in which this organism was isolated later in the life of the plant. Plating resulted in re-isolation of the organism from plants grown either on sterilized soil or on unsterilized soil, in the species *Agropyron elongatum*, *A. repens*, *Bromus inermis*, *B. ciliatus* and *Avena fatua*. Only the two species of *Bromus* yielded the organism when grown on both sterilized and unsterilized soils. *Hordeum jubatum* showed no infection with *F. graminearum*. *Phleum pratense* was the only species resistant to all the organisms studied under the conditions of the experiment.

Relation of Western Rye Grass to the Amount of Take-all in the Following Wheat Crop

In 1927 the Division of Plant Biochemistry of the Department of Field Crops* at the University of Alberta began an experiment to determine the effect of alfalfa, western rye grass, brome and timothy on wheat yield and quality. Three series were laid out, each series having four replicates of each crop arranged as a Latin Square, to be broken in 1928, 1930 and 1932. The second series was plowed in 1930 and seeded to wheat in 1931. It was noticed that a large amount of take-all was present in the wheat on some plots, and counts of take-all were therefore made in order to obtain an estimate of damage. The average damage due to take-all following western rye grass was 23%, and on the plots of wheat after brome grass only 7%. There was no appreciable damage to the wheat following timothy and alfalfa.

In the third series the western rye grass appeared to be dying out in 1931, and in 1932 so little western rye grass was left that the plots were no longer suitable for their original purpose. By this time they had become over-run with weeds, such as dandelions and numerous grasses (Fig. 2).

As it was considered that the killing of the western rye grass might be due to take-all, roots of this grass from each of the plots were plated out at the end of May, 1932. *O. graminis* was isolated from roots from all four replicate

TABLE VI
INFECTION OF WHEAT WITH TAKE-ALL AND FOOT ROT WHEN GROWN ON
SOILS AFTER DIFFERENT CROPS

Type of plot from which soil was obtained	Number of pots of wheat grown	Average plant height, cm.	Av. % degree infection of wheat with <i>O. graminis</i>	Av. % degree infection with other organisms
Western rye grass	20	26.1	4.8	6.2
Brome	8	26.4	0.0	3.3
Timothy	8	25.1	0.0	4.0
Alfalfa	8	26.9	0.0	4.5
Summerfallow	10	28.5	0.0	5.5
Sterilized soil	10	27.8	0.0	0.9

*Under the direction of Dr. R. Newton.

plots of western rye grass. *H. sativum* was also isolated, but symptoms of injury of plants by *O. graminis* were far more marked than were any other symptoms. No sign of take-all appeared on the brome or timothy plants. In order to determine the abundance of *O. graminis* in the soil from these plots and on soil growing other grasses or being summerfallowed, twenty five-inch pots were filled with soil from the western rye grass plots together with any stubble that may have been in the soil, eight each from the brome, timothy and alfalfa plots, ten from an adjacent summerfallowed soil, and ten with sterilized black soil. All were seeded to wheat, which was harvested after 21 days. Each plant was measured and the infection with *O. graminis* and with other organisms estimated. The data are given in Table VI.

The results summarized in Table VI show that *O. graminis* was isolated only from soil which had previously grown western rye grass. It appeared that in all probability other wheat foot-rotting organisms were also more abundant after western rye grass, but attempts to isolate them were not made. These results are further evidence of the part played by this grass in the problem of foot rot in wheat. The survey made seemed to show that there is a fairly general tendency for the older fields of *A. tenerum* to be more severely damaged by foot-rotting organisms, and it would be of value to determine if this is generally the case.

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A STUDY OF VARIOUS MEASURES OF VISCOSITY OF FLOUR-WATER SUSPENSIONS IN RELATION TO QUALITY¹

BY R. K. LARMOUR² AND H. R. SALLANS³

Abstract

Viscosity measurements were made with the MacMichael viscosimeter on leached and unleached suspensions of 11 experimentally milled flours representing the range 8.2–18.3% protein. The leached flour values gave the least satisfactory differentiation. The actual measurements made on unleached suspensions, or the response to acidulation or to increased flour concentration of the unleached suspensions, gave as much differentiation as the bromate baking test. Of the latter three the simple determination of viscosity of the unleached suspensions is recommended as the most rapid.

Most of the tests of flour quality, with the exception of ash and nitrogen, require a great deal of skill and judgment on the part of the operator. Because these attributes cannot readily be standardized, many modifications of the principal tests have been introduced from time to time and the confusion arising from the variations in the technique of individual workers and the resulting differences in data and in interpretation of data has been the despair of those interested in placing wheat and flour testing on a quantitative basis. The work of Sharp and Gortner (7) in 1923 stimulated interest in the viscosity of flour-water suspensions as a means of estimating flour quality because it seemed possible to subject this method to accurate control and thus establish a test that would be easily replicated by different workers. This, however, was not realized and at present the viscosity test is used very little in routine work. Its disuse is attributable to a number of causes, the principal of which are: first, the method recommended by Sharp and Gortner (7) and later by Johnson and his coworkers (2, 3, 4) involves the leaching of the salts from the flour, and this requires nearly as much time as the baking test, and secondly, the removal of electrolytes makes the colloidal system so sensitive to the influence of conditions that it is difficult to obtain reproducible results. Any method designed to replace the baking test, wholly or in part, must be more rapid or more precise or both; the determination of viscosity of leached flour-water suspensions meets none of these requirements and it is not surprising therefore that it has found little acceptance in routine work.

It seems likely that the baking test, despite its lack of standardization and the great number of slightly understood factors that affect it, will remain, for some time to come, the criterion by which other tests of quality must be judged. Modifications of the baking test have been proposed from time to

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time by various investigators but it is extremely difficult to produce evidence that one procedure or formula is better than another. About the only possible basis on which baking procedures can be compared is "commercial valuation" and as this factor cannot be accurately measured there are always certain to be differences of opinion as to how well a given formula interprets the millers' and bakers' estimates of flour values.

In dealing with western Canadian wheat, the main problem is to estimate the potential strength of flours rather than their value for bread-making *per se*, because most of this wheat is exported to be used in blends with softer wheats and its real worth depends mostly on ability to make up quality deficiencies. During the past few years a number of extensive studies of various baking formulas have been made with particular attention directed to the effect of potassium bromate. Geddes and Larmour (1) discussing the available evidence concluded that the bromate formula is much superior to the basic formula of the A.A.C.C., especially when applied to the evaluation of Canadian hard red spring wheat. In view of the fact that most of the earlier work on viscosity had been referred to baking results obtained by the basic formula it was thought advisable to check the viscosity method against the bromate baking method to see how closely the results were correlated. It was particularly desirable to learn how closely viscosity of unleached flour suspensions would prove to be related to the baking results by the bromate formula.

Material

The material chosen for this study consisted of 11 composite samples of flour experimentally milled from pure strain Marquis of the 1929 crop. These flours had a protein range of 8.2 to 18.3% and were quite representative of flours from the Saskatchewan hard red spring wheat crop of that season. The number of samples in each composite flour, the protein and ash contents, and the more important baking data are given in Table I.

A few words need to be said here regarding the composition of this series. In connection with another study samples of pure strain Marquis were collected from representative places in the province. After milling and baking tests had been made on the individuals, the residues of flour were combined into these 11 samples, the only selection exercised being that of putting flours of about the same protein content (using 1% ranges) into the same lot. At the extremes of the series the number of samples was, of course, rather small, but for protein values of 10-16% inclusive there were not fewer than 11 individual flours in each composite. Using this same series Larmour and Brockington (6) showed that the baking data of the composites corresponded very closely with the averages of the results obtained with the individuals. It was concluded therefore that little, if any, complementary effect occurred as a result of the mixing.

Attention should be directed to the fact that whereas there is a very wide range of protein values, the simple or basic formula gave relatively small differentiation of the samples, while the bromate formula gave results in

nearly the same order as the protein content. As far as we can appraise it, "commercial values" for blending purposes are in about the same order as protein. This is partially confirmed by the fact that baking results with a soft-flour-blend formula fall in this order also.

Experimental

The viscosity measurements were made with the new type MacMichael viscosimeter using a No. 30 wire, a 2-cm. bob, and the 5-cm. bowl revolving at 100 r.p.m. All data are recorded in degrees MacMichael and for sake of brevity these values will be referred to as the viscosity.

TABLE I
ANALYSES AND BAKING DATA OBTAINED WITH THE COMPOSITE MARQUIS SERIES

Flour no.	No. of samples in composite	Protein of flour, %, 13.5% mb.	Ash in flour, %, 13.5% mb.	Simple formula		Bromate formula	
				Loaf vol., cc.	Score	Loaf vol., cc.	Score
1	6	8.2	0.53	505	60	495	66
2	5	9.2	0.53	515	68	525	77
3	11	10.0	0.52	543	75	568	88
4	15	11.1	0.44	573	87	618	100
5	12	12.1	0.51	580	87	670	112
6	28	13.3	0.45	583	86	725	124
7	22	14.0	0.46	625	94	735	126
8	14	15.0	0.54	628	92	815	140
9	12	16.0	0.52	580	84	770	131
10	8	17.1	0.51	560	77	800	137
11	5	18.3	0.58	618	90	865	149

Two series of determinations were made, one on unleached and the other on leached flour-water suspensions, using four concentrations of flour in each. In the unleached series four concentrations of lactic acid were used on each sample, namely 0.5, 1.5, 2.5, and 5.0 cc. of 20% acid per 100 cc. of flour suspension. In the leached series, concentrations of 0.1, 0.3, and 0.5 cc. of 20% acid per 100 cc. of suspension were used. The unleached flour suspensions were prepared by adding 100 cc. of carbon dioxide-free distilled water to the weighed sample in a 250-cc. Erlenmeyer flask and shaking at five-minute intervals for one hour. The leached flour-suspensions were prepared by adding one litre of carbon dioxide-free distilled water to the weighed sample in a one-litre Erlenmeyer, shaking vigorously at 5-min. intervals for 45 min., allowing them to settle for 15 min. and decanting the supernatant liquid; this was repeated twice, but in the second and third extractions the flasks were shaken for only 15 min. and the suspension allowed to settle for 15 min. All work was done in a constant temperature room at 24.5° C.

In making determinations with the various acid concentrations the acid was added in successive increments. For example, with flour No. 1, 26 gm.

per 100 cc., Table II, after the sample had been soaked for one hour, 0.5 cc. of 20% lactic acid was measured into the flask and after thorough shaking, the suspension was poured into the viscosimeter bowl and the reading taken; then an additional 1 cc. of lactic acid was run into the bowl and stirred by means of a glass rod while the bowl was rotating. After two minutes the reading "26° MacMichael" was obtained. This is recorded as the value for 1.5 cc. of lactic acid. After the addition of another 1 cc. of acid the reading "31° MacMichael" was obtained and recorded for 2.5 cc. of acid. This procedure appeared justified by the results of a rather extensive preliminary investigation which showed that with unleached suspensions, the values obtained by adding in a single dose or by increments were practically identical and that with leached samples, although the single dose usually gave higher values, the viscosity-acid curves were nearly parallel except with low concentration of acid in which case they tended to coincide.

TABLE II

VISCOSITY (EXPRESSED IN DEGREES MACMICHAEL) OBTAINED WITH UNLEACHED FLOURS OF THE COMPOSITE MARQUIS SERIES

Flour no.	Weight of flour used, gm.	Cc. of 20% lactic acid added				Flour no.	Weight of flour used, gm.	Cc. of 20% lactic acid added			
		0.5	1.5	2.5	5.0			0.5	1.5	2.5	5.0
		Viscosity readings, degrees MacMichael						Viscosity readings, degrees MacMichael			
1	17	8	9	10	13	7	17	37	48	52	59
	20	12	15	17	19		20	40	67	81	89
	23	16	21	24	27		23	48	93	105	116
	26	21	26	31	35		26	60	144	156	177
2	17	16	20	22	18	8	17	42	48	55	63
	20	20	26	28	25		20	42	70	82	101
	23	26	35	44	41		23	51	106	127	141
	26	31	55	66	62		26	50	141	164	186
3	17	17	22	24	27	9	17	28	47	59	72
	20	23	29	34	40		20	34	72	91	106
	23	28	42	46	52		23	43	108	132	152
	26	29	59	68	76		26	46	159	186	209
4	17	19	26	30	35	10	17	28	61	74	66
	20	24	40	48	56		20	32	86	112	124
	23	29	56	68	81		23	37	124	156	178
	26	35	70	94	108		26	44	172	209	231
5	17	17	32	35	42	11	17	38	63	82	90
	20	37	53	55	60		20	49	106	124	139
	23	38	71	75	88		23	59	143	164	186
	26	47	98	112	126		26	62	190	221	247
6	17	31	38	46	52						
	20	35	56	71	85						
	23	41	85	103	122						
	26	44	110	126	153						

NOTE:—100 r.p.m.; No. 30 wire; 2-cm. bob; 5-cm. bowl; room temperature, 24.5° C.

TABLE III

VISCOSITY (EXPRESSED IN DEGREES MACMICHAEL) OBTAINED WITH LEACHED FLOURS OF THE COMPOSITE MARQUIS SERIES

Flour no.	Weight of flour used, gm.	Cc. of 20% lactic acid added			Flour no.	Weight of flour used, gm.	Cc. of 20% lactic acid added		
		0.1	0.3	0.5			0.1	0.3	0.5
		Viscosity of flow, degrees MacMichael					Viscosity of flow, degrees MacMichael		
1	11	32	35	33	7	11	110	125	117
	14	60	68	68		14	240	245	242
	17	125	130	122		17	265	330	320
	20	155	170	160		20	455	530	510
2	11	52	52	48	8	11	130	150	145
	14	98	102	95		14	210	252	241
	17	165	170	160		17	307	362	350
	20	230	252	232		20	337	342	331
3	11	68	69	62	9	11	115	151	149
	14	125	125	120		14	147	253	258
	17	197	222	235		17	302	397	385
	20	286	340	340		20	430	525	517
4	11	82	91	86	10	11	160	180	179
	14	147	158	160		14	222	274	272
	17	300	308	285		17	326	422	428
	20	357	390	370		20	389	557	549
5	11	95	99	90	11	11	150	184	185
	14	182	192	178		14	243	295	296
	17	307	340	325		17	325	417	419
	20	440	462	470		20	473	567	551
6	11	130	138	127					
	14	225	245	262					
	17	312	392	396					
	20	445	465	455					

NOTE:—100 r.p.m.; No. 30 wire; 2-cm. bob; 5-cm. bowl; room temperature, 24.5° C.

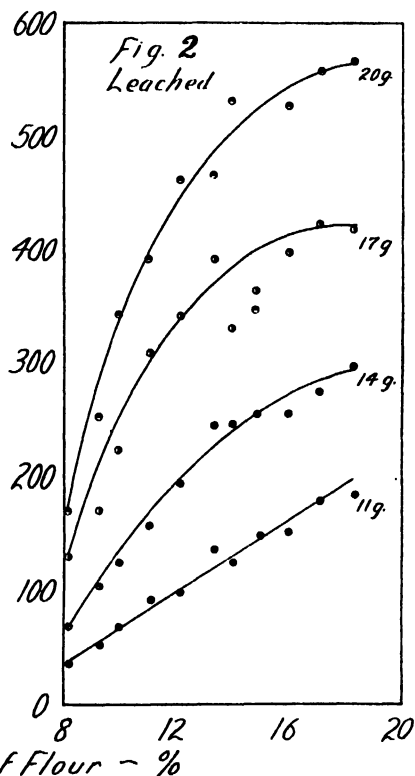
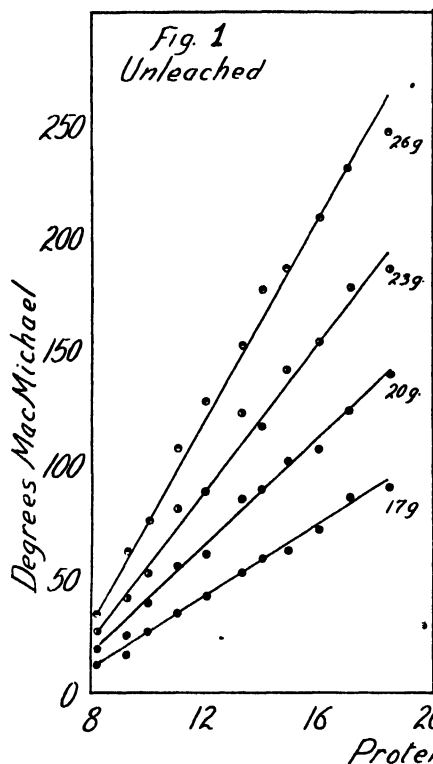
Discussion of Data

The data obtained with the unleached and leached suspensions are given in Tables II and III respectively. As four variables, namely, viscosity, protein, flour concentration and acid concentration are involved it is somewhat difficult to see their relation from the tables and it will be more convenient therefore to discuss the graphs.

Relation of Viscosity and Protein Content

In Figs. 1 and 2 the viscosities are plotted against protein content of flour using the values obtained with 5 cc. and 0.5 cc. of lactic acid solution on the unleached and leached samples respectively. With the unleached samples the relation of viscosity to protein was linear for all concentrations used, whereas with the leached flours only the lowest concentration, 11 gm., gave a linear relation, the other three being decidedly curvilinear. This curvili-

nearity, which is more pronounced at higher than at lower flour concentrations, means that the low-protein flours were more highly differentiated by the viscosity values than the high protein flours. Furthermore, all the curves in Fig. 2 tend to be linear for the lower values. It appears reasonable therefore to assume that the curvilinearity of these graphs is due to errors involved in trying to measure the highly concentrated, high-protein suspensions. At values of about 250° MacMichael and greater, the suspensions get very thick and in all probability are quite plastic. Suspensions for which values of 500° MacMichael are recorded show little tendency to flow and it seems very likely that they have a sufficiently high plasticity to render the readings obtained by the torsion instrument quite invalid.



FIGS. 1 AND 2. Relation of protein of flour and viscosity of unleached and leached suspensions.

The interesting point brought out by these graphs is that when dealing with flour concentrations so low that the maximum viscosity does not exceed about 250° MacMichael, one observes a linear relation between protein content and viscosity with both leached and unleached flours. It should be noted too that the curve for the 11-gm. leached flour concentration practically coincides with that for the 23-gm. unleached flour concentration, using, of course, different acid dosages in the two cases. In other words, by selecting a certain flour and acid concentration, it was possible to get exactly the same

results with unleached as with leached flours. It should be added here that determinations made on leached flours of 10- and 12-gm. concentrations respectively also gave results that showed a perfectly linear relation to flour protein.

Relation of Viscosity to Flour Concentration

In studying the relation of flour concentration to viscosity it has been customary to consider the logarithms of the two variables rather than the actual values, because the latter usually give curves of the exponential type. Sharp and Gortner (7) found that the tangent of the angle made by the logarithmic curve with the axis of abscissa was related to the strength of the flour. This constant, designated b , was calculated by the method of least squares and also by estimation from the plotted values. Data for the different samples are given in Table IV and the curves are shown in Fig. 3.

TABLE IV

COMPARISON OF GORTNER'S CONSTANT b AS OBTAINED FROM THE GRAPHS AND BY CALCULATION, USING LEACHED AND UNLEACHED FLOURS

Flour no.	Unleached flours				Leached flours			
	2.5 cc. 20% lactic		5.0 cc. 20% lactic		0.3 cc. 20% lactic		0.5 cc. 20% lactic	
	Observed	Calcd.	Observed	Calcd.	Observed	Calcd.	Observed	Calcd.
1	2.900	2.766	2.333	2.325	2.762	2.684	2.739	2.645
2	2.900	2.610	2.737	2.922	2.652	2.599	2.500	2.599
3	2.650	2.390	2.444	2.338	2.650	2.658	2.800	2.888
4	2.500	2.649	2.600	2.636	2.583	2.487	2.474	2.513
5	2.500	2.649	2.588	2.571	2.692	2.566	2.750	2.803
6	2.350	2.390	2.500	2.571	2.042	2.026	2.615	2.132
7	2.450	2.494	2.600	2.468	2.400	2.309	2.448	2.349
8	2.501	2.584	2.588	2.519	2.033	1.480	2.233	1.487
9	2.650	2.675	2.545	2.519	2.120	2.086	2.080	2.072
10	2.450	2.442	2.300	2.364	1.862	1.908	1.913	1.908
11	2.300	2.545	2.350	2.312	1.875	1.875	1.800	1.816

Considering first the unleached series, it appears that the value b is practically constant throughout the series of flours. This can be readily observed from Fig. 3 in which the curves are nearly parallel, necessitating a constant value for b . None of the individual values were significantly differentiated from the mean for the series; consequently we can consider that the slopes of the curves were constant. According to the postulate of Sharp and Gortner the value of b is a measure of a qualitative factor in the strength of flour proteins. Assuming their deduction regarding the significance of constant b one would be forced to conclude that no qualitative differences exist in the present series and that the strength of the flours is a direct function of the quantity of protein present.

Using protein of flour as a measure of strength, which according to the data given in Table I appears justifiable, it is evident that there is little relation

to the values of constant b . The correlation coefficient for these two factors was found to be $r = - .309$, which is not significant. As a means for differentiating the unleached flours constant b was of no value.

With leached flour-water suspensions somewhat different conclusions were reached. It can be seen that there was a tendency towards decrease in slope of the logarithmic curves as the protein of the flour increased. This is definitely shown by the values of constant b given in the lower half of Table IV. The values of b for 0.5 cc. of lactic acid correlated with protein of flour give the value $r = - .88$ indicating an inverse relation between these two variables. This would indicate that the relative quality of the protein decreases as the quantity increases. Obviously this is incompatible with the conclusion reached with unleached flour-water suspensions.

In the previous discussion of the data on which these values of b were based, it was pointed out that at a flour concentration of 13 gm. per 100 cc. or greater, the viscosity-protein relation is curvilinear and that this curvilinearity becomes accentuated with increasing flour concentration. Consequently the values obtained on this leached series for constant b are of extremely doubtful value as a criterion of flour strength. It is suggested that viscosity readings higher than 250° may be unreliable, due to the plastic nature of the suspension. If this is true it is conceivable that in the lower viscosity ranges for leached samples the results might be similar, if not identical, for unleached suspensions of some particular flour concentration. In Table V

are shown the data for the 11-gm. leached suspensions using 0.5 cc. of acid, and for the 23-gm. unleached suspensions using 5.0 cc. of acid. It will be observed that the values are in such close agreement that they might easily represent duplicates. Evidently, by employing appropriate concentrations of acid and flour virtually the same results can be obtained by either method. This being the case there appears to be no justification for employing the laborious and time-consuming procedure of leaching the salts from the flours prior to determining the viscosity.

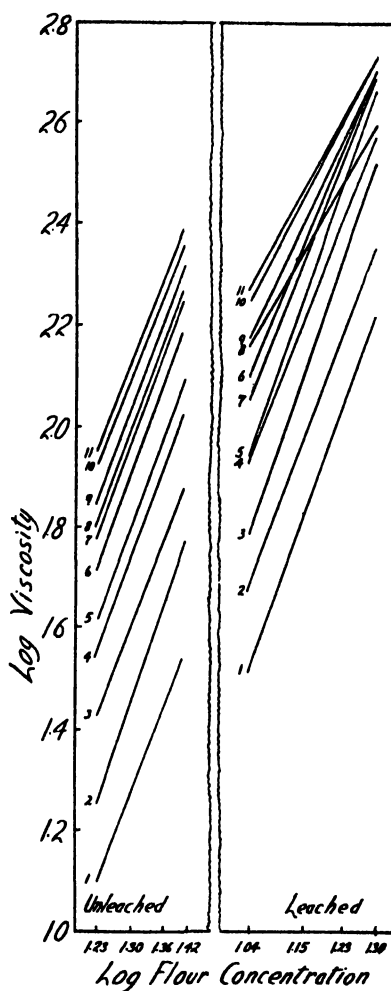


FIG. 3. Comparison of unleached and leached suspensions.

TABLE V

COMPARISON OF VISCOSITY OF UNLEACHED 23-GM. SUSPENSIONS AND LEACHED 11-GM. SUSPENSIONS AT THEIR RESPECTIVE OPTIMUM ACID CONCENTRATIONS

Flour no.	11 Gm. leached, 0.5 cc. 20% lactic	23 Gm. unleached, 5 cc. 20% lactic	Flour no.	11 Gm. leached, 0.5 cc. 20% lactic	23 Gm. unleached, 5 cc. 20% lactic
	Viscosity, degrees MacMichael			Viscosity, degrees MacMichael	
1	33	27	7	117	116
2	48	41	8	145	141
3	62	52	9	149	152
4	86	81	10	179	178
5	90	88	11	185	186
6	127	122			

Relation of Viscosity to Baking Value

Throughout this discussion reference has been made to protein of the flour as the index of baking quality. This was done because in the series studied the relation of loaf volume and general baking quality to protein content was very close. This can be seen from the data in Table VI. It is well known that two variables, each correlated to a common variable, will be correlated themselves. Therefore, because loaf volume (bromate method) and protein showed a correlation of $+0.976$ it follows that any series of values closely related to protein must also be closely related to loaf volume.

TABLE VI

FLOUR PROTEIN, LOAF VOLUME, AND VARIOUS VISCOSITY MEASUREMENTS

Flour no.	Protein of flour	Loaf volume, cc.		Constant <i>b</i> (observed)		Approx. slope of viscosity - flour conc. curve	Approx. slope of viscosity - acid conc. curve, 26 gm. flour	Observed viscosity values	
		Basic bromate		Un-leached	Leached			26 gm. flour, 5 cc. acid, unleached	11 gm. flour, 0.5 cc. acid, leached
1	8.2	505	495	2.33	2.74	22	14	35	33
2	9.2	515	523	2.74	2.50	44	31	62	48
3	10.0	543	568	2.44	2.80	49	47	76	62
4	11.1	573	618	2.60	2.47	73	73	108	86
5	12.1	580	670	2.59	2.75	84	79	126	90
6	13.3	583	725	2.50	2.62	101	109	153	127
7	14.0	625	735	2.60	2.45	118	117	177	117
8	15.0	628	815	2.59	2.23	123	136	186	145
9	16.0	580	770	2.54	2.08	137	163	209	149
10	17.1	560	800	2.30	1.91	145	187	231	179
11	18.3	618	865	2.35	1.80	157	185	247	185

The viscosity data which may be considered as possible criteria of flour quality are: (1) the actual measurements as obtained by either the leached or unleached suspension; (2) the constant *b*; (3) the response to acidulation of the unleached suspensions; (4) the increases of viscosity due to increased

flour concentration. These data, together with protein and loaf volumes, are collected in Table VI. The correlations between these variables are shown by the coefficients given in Table VII.

It should be borne in mind that this series of flours was not a selection of individual samples chosen from the regression of loaf volume (bromate) on protein, but a series of composites made up on the basis of protein without regard to the baking results previously obtained on the individual samples.

TABLE VII

COEFFICIENTS OF CORRELATION FOR THE VARIABLES GIVEN IN TABLE VI

Variables	
Protein x loaf volume (basic)	+ .733
Protein x loaf volume (bromate)	+ .976
Protein x constant <i>b</i> (unleached flours)	— .309
Protein x constant <i>b</i> (leached flours)	— .876
Protein x approx. slope of viscosity-flour conc. curve (unleached, 5 cc. acid)	+ .992
Protein x approx. slope of viscosity-acid conc. curve (unleached 26 gm.)	+ .994
Protein x actual readings on unleached suspensions, 26 gm. flour, 5 cc. acid	+ .997
Protein x actual readings on leached suspensions, 11 gm. flour, 0.5 cc. acid	+ .991
Loaf volume (bromate) x constant <i>b</i> (unleached flours)	— .221
Loaf volume (bromate) x constant <i>b</i> (leached flours)	— .791
Loaf volume (bromate) x approx. slope of viscosity-flour conc. curve (unleached)	+ .981
Loaf volume (bromate) x approx. slope of viscosity-acid conc. curve (unleached)	+ .963
Loaf volume (bromate) x actual readings on unleached suspensions; 26 gm. flour, 5 cc. acid	+ .978
Loaf volume (bromate) x actual readings on leached suspensions; 11 gm. flour, 0.5 cc. acid	+ .977

The close relation between protein and bromate loaf volume therefore cannot be considered fortuitous. As previously pointed out by Larmour (5) the relation between basic loaf volume and protein is curvilinear in the higher protein ranges, while with the bromate loaf volume it is essentially linear throughout the range. On this basis it seems justifiable to use the bromate loaf volume as the criterion by which to judge the usefulness of the viscosity data.

The increased viscosity due to increased flour concentration, and to increased acid concentration, the actual viscosity measurements made on 26 gm. unleached flour suspensions with 5 cc. of acid, and on 11 gm. leached flour suspensions with 0.3 cc. acid, and the protein of the flour were all highly and about equally correlated with bromate loaf volume and with each other. The constant *b* obtained with the unleached suspensions was not significantly correlated with any of the other variables. Constant *b* obtained with leached suspensions was highly correlated negatively with loaf volume and protein. If Sharp and Gortner's interpretation of the meaning of constant *b* is correct it follows that the quality of these flours is constant, if the data from the unleached flours is taken, and this figure therefore cannot be used to differentiate the flours in such a series.

Of the other measurements, the simple determination of viscosity of an unleached suspension is obviously the most rapid and as it gives as much differentiation as any of the others, is to be preferred.

In conclusion it should be pointed out that this study was conducted with sound samples of one variety grown in a single season and although the conclusions reached are well supported by the data, there is no indication of what may be expected with different varieties, different seasons or various forms of damage. This is to be the subject of further investigation.

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A MICROBIOLOGICAL STUDY OF PODSOL SOIL PROFILES¹

BY P. H. H. GRAY² AND N. B. McMASTER³

Abstract

Microbiological studies of samples from the separate horizons and from different depths of soils of the Appalachian upland podsol group show that the activity of the micro-organisms is dependent upon the organic-matter relations in the horizons. The organic-matter horizon is biologically the most active, as shown by analyses for carbon dioxide, nitrate nitrogen, numbers of bacteria, and production of ammonia from urea. Evidence is submitted that the reduced activity of the leached layer and the horizons of accumulation is not due to toxic compounds produced by leaching of the organic matter.

Characteristic profiles of virgin podsol soils in the province of Quebec have been recently described (13, 14). The chief factor that brings about the partition of the soil profile into easily recognizable horizons appears to be the accumulation of a surface mulch of raw or semi-decomposed organic matter that holds moisture and, owing to its acid nature, is the cause of the chemical displacement of basic fertility elements to lower depths of the soil by leaching. In the presence of this high content of potential energy and body-building material the micro-organic population of the virgin soils seems to be at a low level of activity in comparison with that of soils of other groups. Agricultural soils of the upland podsol group that have been cultivated for many years are characterized by their light brown color, in spite of their great content of organic matter, and by their lack of fertility except with continual applications of organic matter and mineral fertilizer. Analyses of three typical cultivated soils show an average total organic carbon of 3.96%, and an average total nitrogen of 0.27% in the surface six inches. Infertility, indeed, as pointed out by Bizzell (4), may be associated with a high content of organic matter in cultivated soils. It has been a matter for discussion whether the lower level of activity in these soils is due to the higher hydrogen ion concentration, the removal of basic nutrients, or to some other cause, such as the accumulation of compounds toxic to the micro-organisms more directly concerned in soil fertility.

The removal of basic nutrients to lower levels of soil suggested that the bacterial flora concerned in reactions important in plant nutrition would be adversely affected either by lack of food material or by the production of acidic or other substances toxic to these organisms. It seemed, therefore, to be of first importance to analyze the distinctive horizons in virgin soils of this nature, to ascertain their biological activity and some at least of the factors affecting that activity. Such studies would appear to be of greater value if a limit having some reference to field practice were set to the depth

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of soil from which samples should be taken. In the studies previously made certain biological aspects of the surface eight inches of virgin soils were reported. In a sample of that depth the proportions of the different horizons vary in different soils; variation in thickness of horizons also occurs in sub-samples of the same soil (see Fig. 1). In the present work a depth of 12 in. was chosen, in order to include part at least of the horizon that receives a large proportion of the leached constituents. This limit of depth for sampling had a certain value in that the soils selected for this study adjoined cultivated soils of the same type in which experiments were in progress to ascertain the effects of ploughing to a greater depth than was customary. Deep ploughing has been recommended (14, 15) as a remedial practice for recovering the lost fertility of soils of this nature. Whether the biological action on the organic matter is at a higher level in the cultivated soils has not yet been ascertained.

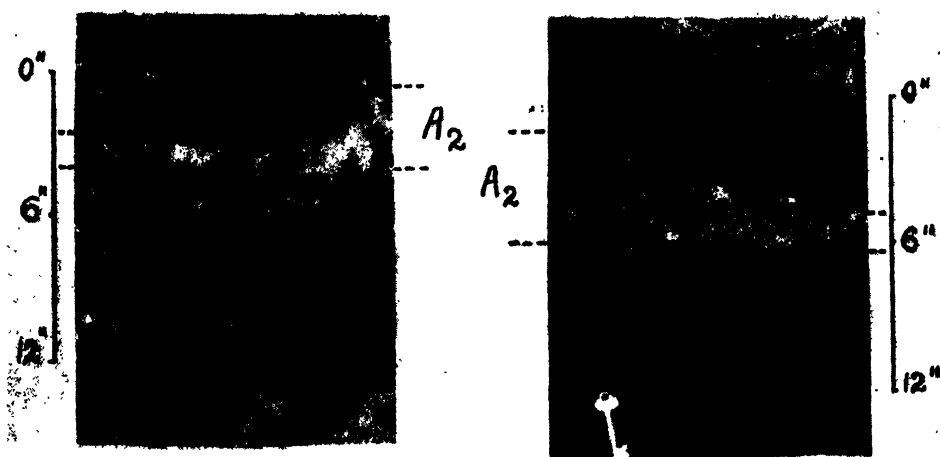


FIG. 1. Representative profiles of upland podsol soils. The thicknesses of the A_2 (leached) horizons and the depths of the profiles are indicated.

The present paper is an introduction to the study of the biological conditions in the horizons of representative virgin soils of the Appalachian upland podsol group. In addition, studies have been made upon the biological activity of freshly mixed horizons to depths of 6 and 12 in., to determine the relative activity in soil to these depths and to provide a basis for comparison with the same soils under cultivation.

Soils and Soil Sampling

The virgin soils selected for these studies were three from the Eastern Townships region of Quebec, that were adjacent to cultivated soils in the same area upon which investigations of a similar nature were in progress. A description of the soil profiles is given in Table I.

TABLE I
DESCRIPTION OF SOIL PROFILES

Sample	Ecology	Depths of horizons, and description
S	Permanent pasture, 1250 ft. above mean sea level; near maple bush; moss hummocks prevalent	A ₁ , 1-2 in., dark brown humus and semi-decomposed residues A ₂ , ½-3 in., ashy-grey to dark grey leached fine sandy loam B, 3-6 in., reddish brown sandy loam, shading to yellowish brown sandy loam, with some stones, down to 12 in.
R	Permanent pasture, 675 ft. above mean sea level; <i>Thuja</i> and other evergreens; moss hummocks	A ₁ , 1-2½ in., dark brown to black mucky fine sandy loam A ₂ , 1-1½ in., ashy leached fine sandy loam B, 5-6 in., reddish brown fine sandy loam, shading to greyish brown fine sandy loam down to 12 in. About 1 in. of the C horizon is included in B.
B	Woodlot, 675 ft. above mean sea level; mixed coppice, birch predominant	A ₁ , 3-5 in., dark chocolate-colored fine sandy loam A ₂ , 1-1½ in., ashy leached fine sandy loam B, 1-3 in., reddish brown fine sandy loam, shading to light reddish yellow fine sandy loam at 12 in.

In the above description no attempt is made to differentiate between sub-horizons of the B (alluvial) horizon.

Samples were taken at different seasons during 1931, as shown in the succeeding tables of results. Samples were obtained from the separate horizons by means of a flat spade, the final separation being done by hand. Horizon B was sampled down to a depth of 12 in. from the surface. To obtain samples of the two depths, the soil was sliced vertically to the required depth, and different slices thoroughly mixed by hand.

Analytical Methods

Moisture. Moisture in the fresh samples and hygroscopic moisture in air-dried samples were determined by the standard methods of the Association of Official Agricultural Chemists (1). The water-holding capacity of the air-dried samples was determined by the method previously described (13); tests were made to compare this method with that described as the "box" method (11), and close agreement was obtained; the latter method was found, however, to be too lengthy a procedure for the number of samples involved. The degree of saturation of the fresh soil, on a basis of moisture-free soil, was calculated from the equation

$$s(\%) = \frac{m - h}{c} \times 100,$$

in which *m* is the moisture in fresh soil, *h* is the hygroscopic moisture and *c* is the water-holding capacity of air-dried soil. The moisture relations, together with the loss on ignition, are shown in Table II. All results, in this and later tables, are reduced to a basis of moisture-free soil.

Hydrogen ion concentration. The pH of fresh samples from the three horizons was determined by the bubbling hydrogen electrode, using 3.5 gm. of air-dried soil in 12.5 cc. of distilled water.

TABLE II

MOISTURE AND LOSS ON IGNITION IN SAMPLES FROM SOIL HORIZONS AND DIFFERENT DEPTHS (EXPRESSED AS PERCENTAGES ON A BASIS OF MOISTURE-FREE SOIL)

Soil	Date sampled 1931	Horizon	Moisture in fresh sample, %	Hygroscopic moisture, %	Loss on ignition, %	Water-holding capacity, %	Moisture in fresh sample, as per cent of water-holding capacity
S	Oct. 23	A ₁	97.38	8.09	42.38	101.50	87.89
	Oct. 23	A ₂	18.74	0.82	2.07	37.52	65.12
	Oct. 23	B	34.97	3.84	7.93	47.77	65.18
R	Sept. 29	A ₁	207.50	12.14	60.16	152.50	128.10
	Sept. 29	A ₂	34.41	2.44	5.85	51.74	61.80
	Sept. 29	B	52.41	3.02	5.96	58.20	84.86
S		Depth, in.					
	Aug. 4	0-6	44.73	3.67	10.19	63.75	64.40
	Aug. 4	7-12	31.69	3.15	7.19	50.81	56.16
	Sept. 15	0-6	23.25	3.26	5.48	50.59	39.52
	Sept. 15	0-12	24.69	2.41	4.90	49.16	22.82
R	Aug. 18	0-6	43.53	3.67	14.05	57.80	68.98
	Aug. 18	0-12	29.51	2.56	6.61	48.20	61.51
B	July 21	0-6	84.78	4.65	18.31	72.04	111.30
	July 21	0-12	70.89	4.05	14.72	66.06	101.30

Loss on ignition and organic carbon. These were determined by dry combustion methods (14).

Total nitrogen. Total nitrogen was determined by the Gunning-Hibbard method as described by the Association of Official Agricultural Chemists (1).

The results of these determinations are shown in Tables II and III, as percentages on a basis of moisture-free soil; each value in these tables is a mean of duplicate determinations. The analyses which compose Table III were carried out in the Chemistry Department, Macdonald College.

Carbon dioxide. The evolution of carbon dioxide from fresh samples was determined as previously described (13). Usually, 500 gm. of soil was

TABLE III

PHYSICAL AND CHEMICAL CHARACTERISTICS OF SAMPLES FROM SOIL HORIZONS

Soil	Horizon	pH	Carbon (organic), %	Total nitrogen, %	Ratio C : N
S	A ₁	5.23	17.060	0.666	25.6
	A ₂	4.63	0.566	0.039	14.5
	B	5.15	3.468	0.190	18.3
R	A ₁	5.10	31.340	1.308	23.9
	A ₂	5.25	0.617	0.033	19.0
	B	5.46	2.208	0.143	15.4

placed in the container, and the gas collected at intervals for periods varying from 286 to 400 hr. Tests were also made for variation among triplicate subsamples, and agreement was found to be close in the case of eight samples. The results are expressed as mgm. of carbon dioxide per 100 gm. of moisture-free soil per 100 hr.

Nitrate nitrogen. Nitrate nitrogen was determined by Harper's modification of the phenol-disulphonic acid method (10). The soils were incubated with the original moisture at room temperature, in glass jars that permitted aeration but allowed a minimum of evaporation. The results, which represent nitrification of the soil's own nitrogen, have been expressed as parts per million of moisture-free soil. A test for variation was made with triplicate subsamples of one sample, and close agreement was found.

Bacterial numbers. Bacteria and actinomycetes in fresh samples were counted by the plate method, using Thornton's medium (16). A set of five plates was prepared from each sample; in some cases, however, the numbers could be calculated only from a smaller number of plates; the number of plates used, and the reliability of the results in relation to the χ^2 index of dispersion, are shown in the tables of results (Tables VI and XI).

Soil Horizon Extract Media

Soil solution was extracted from the three horizons A₁, A₂ and B, of soil S by means of a displacement method. The samples from each horizon were dried and the water-holding capacity of each was determined. They were then remoistened to about 60% of the water-holding capacity, and packed with gentle but firm pressure into percolation cylinders, in which they were allowed to stand for a short period. Distilled water was then used to form a head of pressure, 3 to 4 in. deep, above the moist soil. The first few cubic centimetres of displaced liquid was discarded, and an amount of soil solution equal in volume to about 75% of the calculated true solution was collected. Clear light-amber colored liquids were thus obtained from each of the three horizons. With these extracts as the solvents, three agar media were prepared from each; to one portion nothing was added except the agar; to a second 0.1% of mannitol was added as a source of carbon; and to a third 0.05% of potassium nitrate was added as a source of nitrogen. A sample of soil S was plated on each of these media, and on Thornton's count medium. In the dilution fluid sufficient phosphorus, as dipotassium hydrogen phosphate, was added to give to the extract media in the plates an amount comparable with that in Thornton's medium. The results are shown in Table VII, in which the figures represent the mean number of bacterial colonies in four (in one case, three) plates.

Production of Ammonia from Urea

A mass of air-dried soil equivalent to 5.0 gm. of moisture-free material was inoculated into 50 cc. of a solution consisting of urea, 5.00%, and dipotassium hydrogen phosphate, 0.05% in distilled water in Hansen yeast culture flasks, and the cultures incubated at 25° C. in a room the temperature

of which was controlled by a thermostat. The amount of ammonia was titrated at intervals with *N*/14 sulphuric acid, using methyl red as the indicator. The results, expressed as mgm. of ammonia nitrogen per cc. of culture, are given in Tables VIII and XII. Ammonia found in an uninoculated flask of the same medium amounted to 0.61 mgm. per cc. This has been subtracted to give the values quoted.

Discussion of Results

A. The Separate Horizons

The biological activity of the separate horizons is shown by the amount of carbon dioxide evolved to be greatest in the organic-matter horizon of both soils of which the separate horizons were studied (see Table VI). The ratio of the amount evolved by the *A*₁ horizon to that evolved by the leached

layer is in the order of 25 to 1 in both soils. There is little or no difference between the leached horizon and that below it.

The results of the analyses for nitrate nitrogen in the separate horizons are shown in Table V. No nitrate

TABLE IV
CARBON DIOXIDE EVOLUTION; SAMPLES FROM SOIL HORIZONS

Horizon	Carbon dioxide, mgm. per 100 gm. per 100 hr.	
	Soil <i>S</i>	Soil <i>R</i>
<i>A</i> ₁	138.80	358.30
<i>A</i> ₂	5.30	14.20
<i>B</i>	6.10	5.60

TABLE V

NITRIFICATION IN SAMPLES FROM SOIL HORIZONS

Soil	Days	Nitrate nitrogen, parts per million			Soil	Days	Nitrate nitrogen, parts per million		
		<i>A</i> ₁	<i>A</i> ₂	<i>B</i>			<i>A</i> ₁	<i>A</i> ₂	<i>B</i>
<i>S</i>	5	Trace	Trace	Trace	<i>R</i>	7	Nil	Trace	Trace
	24	86	Trace	Trace		29	307.6	17.6	8.7
	209	137.5	99.5	14.0		51	452.2	29.8	12.1
						233	694.4	66.3	23.5

nitrogen was found in any of the fresh samples, and in some cases only traces were found after 24 days of incubation. The activity of the nitrifying bacteria follows the same trend in the three horizons as that of the whole population, in that the organic-matter horizon is considerably more active than those below it. The rate of nitrification in the organic matter horizon of soil *S* fell off rapidly after the 24th day, only 52 p.p.m. of nitrate nitrogen being formed in 185 days, as compared with 100 formed in the leached layer during the same period. Nitrification was rapid in the organic-matter horizon of soil *R*, 307.6 p.p.m. being formed in 22 days, a daily average of 14 p.p.m.; the rate then fell to 6.5 p.p.m. daily during the next 22 days, and to 1.3 p.p.m.

daily between the 51st and the 233rd day. These results are not in agreement with those of Lunt (12), who obtained little or no nitrate nitrogen in the humus horizons of forest podsol soils of New England.

The degree of nitrification in the lower horizons is not proportionate to the amounts of total nitrogen in them.

The numbers of bacteria in the different horizons (see Table VI) appear to follow the same trend as the activity of the whole population and of the nitrifying bacteria. The numbers of bacteria in the leached horizons can be expressed as a percentage of those in the organic-matter horizons. In soil *S*, this percentage was 3.02 and in soil *R* it was 3.44. It is interesting to note that the proportional figures for the evolution of carbon dioxide are 3.8 and 3.9% respectively.

Fraenkel (8) found that the reduction of the bacterial flora with increasing depth in virgin soils was sharply defined. Waksman (18), who gives references to this aspect of soil bacteriology, found that, in a forest soil in the air-dried condition, numbers at arbitrary vertical intervals were progressively less and that there was an association between numbers and the carbon and nitrogen content of the samples. The differences in carbon and nitrogen were, however, considerably less than those reported here (Table III). A comparison of the numbers in the three horizons and their respective carbon and nitrogen contents suggests that there is a direct, though not a close, relation between bacteria and organic carbon.

The numbers of actinomycetes follow the same trend as the bacteria in the three horizons and in the other samples (see Tables VI and XI). The proportion of these organisms, expressed as a percentage of the total plate count, appears to be rather lower than that usually found in cultivated soils when plated on the same medium.

In order to determine if the bacterial numbers in horizons *A*₂ and *B*, as found by plating the fresh soil, were less than those in the organic-matter

TABLE VI
NUMBERS OF MICRO-ORGANISMS IN SOIL HORIZONS

Soil	Date of plating	Horizon	No. of plates used in counting	Mean of bacterial colonies	Index of dispersion	Bacteria per gm.	Actinomycetes per gm.	Actinomycetes % of total
<i>S</i> Oct. 23	Oct. 26	<i>A</i> ₁	3	117.0	Within normal	23,210,000	5,025,000	17.8
		<i>A</i> ₂	3	614.0	Within normal			
		<i>B</i>	1	700.0	Within normal			
<i>R</i> Sept. 29	Oct. 9	<i>A</i> ₁	5	176.0	Within normal	10,116,000	1,494,000	12.9
		<i>A</i> ₂	5	280.0	Within normal			
		<i>B</i>	5	93.4	Excessive			
						464,610	25,890	5.3
						348,000	17,270	4.7
						135,670	10,730	7.3

horizon on account of the proportionate amounts of available nutrients or due to the presence of toxic compounds in the soil solutions, a sample of soil *S* was plated on media prepared with the solutions extracted as described, and on Thornton's medium. The results are shown in Table VII.

TABLE VII
COLONIES ON SOIL HORIZON EXTRACT MEDIA

Extract from horizon	Extract alone	Extract with mannitol	Extract with nitrate
A ₁	98.50	105.70	111.50
A ₂	78.00	65.25	81.00
B	69.75*	61.75*	68.00

Colonies on Thornton's medium, 86.75.

*x² excessive.

The extract from the organic-matter horizon either alone or with added carbon or nitrogen gave higher numbers than the medium used as control, but the difference is found to be not significant when the *t* test for significance is applied (7). The higher numbers of colonies on the extract medium from horizon A₁ with additional carbon or nitrogen are also not significantly different from the number obtained with the extract alone. By the same test there is no significance between the numbers obtained by the extracts from horizons A₁ and A₂ when used alone, but when carbon or nitrogen is added the numbers in the leached horizon are significantly lower in both cases. The numbers shown by the plates from horizon B are not significantly lower than those shown by the plates from the leached horizon. The numbers given by the extracts from the leached horizon and horizon B are also not significantly different from the number given by the control medium. These results would appear to confirm the suggestion that lower numbers in the lower depths of these soils are not due to soluble toxic compounds.

The ammonification of urea is dependent to some extent upon the initial numbers of bacteria, as shown by Drewes (6) and Viehoveer (17), and upon the subsequent rates of multiplication. Thus a sample having fewer bacteria may produce less ammonia in the first few days, but as growth proceeds the numbers of organisms may, provided there is sufficient available carbon, increase to a maximum; the amount of ammonia finally equals that produced in a shorter time by a sample having a greater initial number of bacteria. Comparisons of the amounts of ammonia produced can therefore be best made during the early stages of the reaction, and they may serve as indexes of the availability of the carbon in the soil sample. The results of this test are shown in Table VIII.

The organic-matter horizons are considerably the most active in both soils. The hydrolysis was complete by the 7th day in the case of soil *S*, and by the 15th day in the case of soil *R*. The maximum was not reached in the case

TABLE VIII
AMMONIA PRODUCED FROM UREA; SOIL HORIZONS

Soil	Horizon	Ammonia-nitrogen, mgm. per cc.						
		Days	3	7	9	16	22	41
S	A ₁		20.17	27.15	25.46	24.66	25.28	24.04
	A ₂		1.47	9.99	12.93	22.65	24.41	23.16
	B		1.08	7.40	9.86	15.60	17.36	16.12
R		Days	3	7	9	15	22	41
	A ₁		6.66	24.62	26.44	28.26	25.48	25.79
	A ₂		1.09	6.83	10.47	14.70	13.58	12.59
	B		1.62	6.75	8.70	12.51	14.66	15.23

of either of the leached horizons, or of the B horizons; the difference in activity between these horizons is only slight. These results suggest that soluble compounds of carbon present in the organic matter are in sufficient quantity to allow the bacteria to complete the reaction. The amounts of available carbon are apparently much less in the leached horizons and in the horizons of accumulation; the ammonia is not, however, proportionate to the amount of total carbon.

Viehoever (17) suggested that a urea-decomposing organism *B. probatus*, utilized the compound autotrophically when grown in pure culture, obtaining its carbon from the air and from mineral compounds, including the ammonium carbonate formed from the hydrolyzed urea, hydrolysis being effected by the sterilization of the medium. While many workers are agreed (9) that available carbon compounds stimulate the production of ammonia from urea, Beijerinck (2) and Bierema (3) have shown that soil organisms are not able to use the compound as a source of carbon and nitrogen. The different rates at which the bacteria in the three horizons utilize the compound suggest that the amount of available food material as well as the initial numbers must be effective in determining those rates. In view of the results obtained by plating on the media prepared from the separate horizons, it would appear that the number of bacteria is the chief factor in these different rates of ammonification. The possibility that the differences are due to toxic compounds in the lower layers of soil appears to be ruled out by the results of plating on the extract media.

The solutions resulting from the action of the ammonia on the organic matter in the samples in the culture flasks varied from light brown to dark brown, depending to some extent upon the amount of ammonia present; the depth of color was not, however, directly parallel with the degree of ammonification.

B. Samples from Different Depths

It might be assumed, as indicated previously, that these soils are relatively much richer in the virgin state than they become after cultivation, and that

treatment widely different from that normally practised should be recommended. One of these recommendations is that they should be ploughed to a depth sufficient to return to the soil the basic nutrients removed by leaching, on the assumption that the organic matter would thereby be more readily decomposed. Against this it is widely believed that such treatment results in a low level of fertility.

The studies reported in this part were made to determine the biological activity of samples of soil taken to depths of 6 and 12 in. from the same trenches as those from which samples of the separate horizons were taken. The purpose of these studies was to compare the effects of mixing the organic-matter horizons with different amounts of "mineral soil" from the lower horizons, and thereby to obtain some knowledge of the biological activity of virgin soils in comparison with that of the same soils after many years of cultivation.

TABLE IX
CARBON DIOXIDE EVOLUTION; SAMPLES FROM DIFFERENT DEPTHS

Depth, in.	Soil S	Soil R	Soil B
	Carbon dioxide, mgm. per 100 gm. per 100 hr.		
0-6 (Aug. 4)	40.96 (1)*	—	—
7-12 (Aug. 4)	7.20 (2)	—	—
0-6	22.90 (3)	51.83 (5)	74.53 (7)
0-12	12.80 (4)	22.23 (6)	45.87 (8)

* The values for triplicate subsamples are as follows: (1) 42.3, 40.0, 40.0; (2) 8.0, 6.8, 6.8; (3) 24.7, 22.1, 21.9; (4) 14.7, 12.1, 11.6; (5) 52.4, 52.3, 50.8; (6) 24.2, 22.4, 20.1; (7) 78.2, 73.1, 72.3; (8) 46.2, 45.8, 45.6.

The degree of activity represented by the evolution of carbon dioxide in the upper 6 in. of soil and in the lower 7-12 in. can be compared for soil S only, sampled on Aug. 4. The amount of carbon dioxide in the upper layer is, as shown in Table IX, nearly six times the amount produced by the lower 6 in. of soil. The amount obtained, approximately 41 mgm., from the upper layer can be calculated as that which would be expected on a basis of proportionate amounts of the separate horizons in a sample to the depth of 6 in., in which the horizons do not vary much in thickness from an average of the values shown in the description of the soils. The amount obtained from the 7-12 in. sample, also, is not greatly different from the value obtained in the case of the B horizon, 6.1 mgm., as shown in Table IV.

In comparing the values obtained for the carbon dioxide evolved by the 6 in. and the 12 in. depths of soil, it is clear that dilution of the upper 6 in. with material from the B horizon effects more than a twofold reduction in activity in the case of soils S and R, and a somewhat less reduction in the case of the soil B. The amount evolved by the upper layer of soil R can be considered as approximately what would be expected on a basis of proportionate masses of soil from the horizons.

TABLE X
NITRIFICATION IN SAMPLES FROM DIFFERENT DEPTHS

Soil	Days	Nitrate nitrogen, parts per million		Soil	Days	Nitrate nitrogen, parts per million	
S (Aug. 4)	2	0-6 in.	7-12 in.	R	1	0-6 in.	0-12 in.
	37	nil	nil		23	trace	nil
	50	37.6*	trace		36	18.7	4.0
	107	62.1	13.9		93	33.1	9.4
	289	117.9	22.9		275	117.3	33.9
S (Sept. 15)		159.2	24.5	B		159.2	66.0
	1	0-6 in.	0-12 in.		2	nil	nil
	15	nil	nil		20	trace	trace
	65	trace	nil		64	45.7	32.4
	247	35.7	16.1		121	126.2	93.2
		68.5	29.3		303	178.6	162.8

* In triplicate subsamples: 39.64, 36.67, and 36.41.

In neither soil was there evidence of reduced activity other than that due to dilution.

The amount of nitrate nitrogen was greater in the upper, 0-6 in., samples throughout the period of incubation (see Table X). The lower degree of nitrification by the 0-6 in. sample of soil S, of Sept. 15, in comparison with the 0-6 in. sample of Aug. 4, is probably due to the soil being too dry at the time of sampling. It will be seen in the last column of Table II that the percentage of saturation of the soil sampled on Sept. 15 was 39.5%, while that of the other sample was over 60%. It would seem that drought rather than excessive moisture adversely affects the nitrifying bacteria in soils of this nature.

The results given for soils S (Aug. 4) and R are also shown in Fig. 2, wherein it will be found easier to compare the relative degrees of nitrification in the two pasture soils. There is close agreement between the 0-6 in. samples. Nitrification probably continued for some time after the final test in these two samples and in the 0-12 in. sample of soil R, but it ceased at about the 15th week in the lower (7-12 in.) sample of soil S. The other samples of soil S have been omitted from the graph for the reason stated.

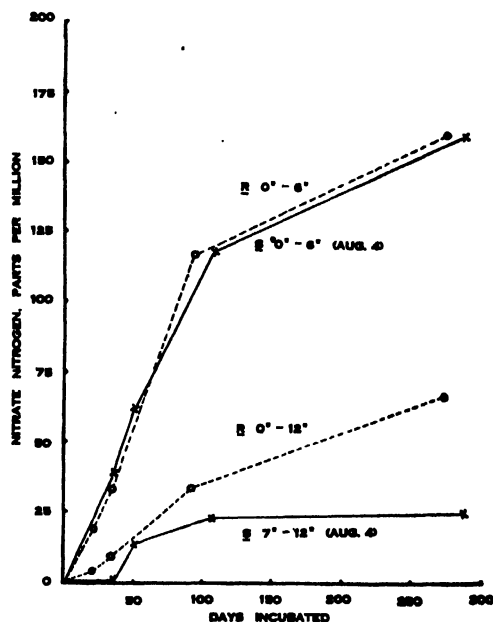


FIG. 2. Nitrification in samples from different depths of virgin podsol soils.

The degree of nitrification in both layers of soil *B* would suggest that, in this soil at least, there are no compounds toxic to these organisms in the lower depth of the sample.

The numbers of bacteria and actinomyces are shown in Table XI.

TABLE XI
NUMBERS OF MICRO-ORGANISMS IN SAMPLES FROM DIFFERENT DEPTHS

Soil; Date	Date of plating	Depth of sample, in.	No. of plates used in counting	Mean of bacterial colonies	Index of dispersion	Bacteria per gram	Actinomyces per gram	Actinomyces, per cent of total
S Aug. 4	Aug. 5	0- 6	4	126.2	Excessive	818,800	163,400	16.6
		7-12	4	23.6	Within normal	145,530	28,370	16.3
						2,788,500	213,500	7.1
S Sept. 15	Sept. 16	0- 6	3	522.0	Excessive	1,296,900	144,100	10.0
		0-12	4	266.0	Within normal	1,546,500	374,500	19.5
						674,300	232,900	25.7
R Aug. 18	Aug. 19	0- 6	5	212.6	Within normal	643,640	86,160	11.8
		0-12	5	94.0	Excessive	970,000	114,800	10.6
B July 21	Aug. 12	0- 6	5	73.3	Within normal			
		0-12	5	108.4	Excessive			

The numbers found in the upper 0-6 in. samples are about twice those in the 12 in. samples in the case of those from soil *S*, of Sept. 15, and soil *R*. The variation shown by the two 0-6 in. samples of soil *S* may be due either to moisture in the soil, or to season. Cobb (5) has shown that numbers of bacteria and actinomyces in virgin forest soils of New York State fluctuate with season, and states that the moisture exercises the greater control. No evidence is available as to seasonal fluctuations in virgin soils under pasture.

The value shown for bacteria in the upper layer of soil *S* (Aug. 4) is not reliable since the value for χ^2 is excessive; the difference, however, between this value and that for the lower 6 in. is sufficiently great to show their approximate relation.

The numbers in the 0-12 in. sample of soil *B* are probably not significantly different from those in the 0-6 in. sample. The bacterial numbers and the nitrifying capacity of these two samples do not greatly differ, although the difference in the evolution of carbon dioxide is quite appreciable.

The production of ammonia from urea is shown in Table XII. The amounts on the third day appear to be comparable with the number of bacteria in the respective samples. The amounts approach equality on the 9th day, except in the case of the 0-6 in. and 7-12 in. samples of soil *S*, in which the amount produced by the upper layer remained higher even at the 22nd day. The lower layer of soil *B* became more active than the upper on the 15th day; the 12-in. sample of this soil, in other respects also, as shown previously, differs little from the 6-in. sample.

TABLE XII

AMMONIA PRODUCED FROM UREA: SAMPLES FROM DIFFERENT DEPTHS

Soil	Depth, in.	Ammonia nitrogen, mgm. per cc.					
		Days	3	7	9	15	22
S Aug. 4	0- 6		3.03	25.70	27.38	27.99	26.11
	7-12		0.57	7.37	11.50	20.63	22.90
S Sept. 15	0- 6		5.11	21.23	26.85	27.33	—
	0-12		2.01	15.41	21.03	27.51	—
R	0- 6		2.21	27.80	27.76	—	—
	0-12		1.25	21.66	28.04	—	—
B	0- 6		1.71	11.20	15.24	20.58	22.71
	0-12		0.96	8.08	13.32	24.20	26.80

General Discussion

The microbiological activity of these podsol soils appears to be controlled by the organic-matter relations of the well-differentiated horizons. The biological activity is almost entirely confined to the mat of organic matter that covers the mineral soil. The data show that the activity of the mineral horizons of the soil through which, and to which, the organic and inorganic materials are leached can be expressed as a small fraction only (less than 4%) of the activity of the surface layer of organic matter. Disturbance of the relative positions of the horizons, during the time covered by the analyses reported above, does not appear to cause any beneficial or harmful results in so far as concerns the aerobic bacteria appearing on a standard plate, or the nitrifying bacteria. The results suggest that the reduced activity in the lower layers of soil is due to position and not to the presence of toxic material removed from the organic-matter horizon by leaching and deposited in the B horizon.

The evidence submitted with regard to the plate counts of bacteria on media prepared with the solutions from the three horizons and the tests for the ammonification of urea by equal masses of soil from the separate horizons and different depths, would appear to confirm these conclusions.

It is perhaps worth while to record here that the activity of these virgin soils, as shown by the evolution of carbon dioxide, is of a higher order than the activity of the adjoining land that has been under normal cultivation for many years (see Table XIII). The closer agreement between these values in the case of soil S may be due to the fact that this has been cultivated for many years to a greater depth than is customary.

Bacterial numbers, on the other hand, are considerably higher after cultivation. These soils have received organic manures and chemical fertilizers since they were originally broken up; this would no doubt account for the increased number of these organisms.

TABLE XIII

COMPARISONS BETWEEN MICROBIOLOGICAL ACTIVITIES OF VIRGIN AND CULTIVATED SOILS (SAMPLES OF 0-6 IN. DEPTH)

Soil	S (Aug. 4)	R (Aug. 18)	B (July 21)
Virgin Cultivated	Carbon dioxide, mgm. per 100 gm. per 100 hr.		
	41	52	75
	47	32	35
	Bacteria and actinomyces, per gm.		
Virgin	980,000	1,920,000	730,000
Cultivated	10,540,000	10,950,000	14,440,000

Summary

1. Microbiological characteristics of representative virgin soils of the up-land podsol group of Quebec are described and discussed.

2. The organic-matter horizon of virgin podsol soils is biologically more active than the leached layer or the horizons of accumulation, as shown by the evolution of carbon dioxide in fresh samples, by nitrification of soil nitrogen, by numbers of bacteria and actinomyces, and by the production of ammonia from urea.

3. The amounts of carbon dioxide evolved and the numbers of bacteria and actinomyces in the leached layers are about 4% of those in the organic-matter horizons in two widely separated soils.

4. The soil solutions from the three horizons studied contain sufficient nutrients for the same number of bacteria appearing on a standard medium.

5. The number of bacteria and the amount of available nutrients in the horizons control to some extent the rate of ammonification of urea in culture solution.

6. Mixture of the organic-matter horizon with soil from the leached layer and from the horizon of accumulation reduces the activities of the micro-organisms to a lower level than that shown by the organic-matter horizon. The reduction in bacterial numbers appears to be due to dilution of the larger population.

7. Evidence is submitted that reduced activities are not due to the presence of toxic compounds.

8. The amount of carbon dioxide evolved from samples of virgin soil is greater than that from the soil after normal cultivation, but the numbers of bacteria in the cultivated soils are considerably greater than those in the virgin soils.

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THE BINOCULAR OSCILLATION AND FUSION OF COLORS¹

BY JOHN F. ALLEN²

Abstract

When pairs of colors fall upon the same area of a retina it is impossible to separate the receptor actions in the retina from those that take place in the visual centres of the cortex. If the separate colors, however, are allowed to fall upon the two eyes, the fusion color effects must be attributed to the sensations in the cortex.

In this investigation the binocular fusion of complementary colors into white is confirmed, as well as the binocular fusion of red and green colors into yellow. The sensation of yellow is therefore proved to be compounded of the fundamental sensations, red and green.

The binocular oscillation or rivalry of colors was specially studied and shown to be due not to fluctuations of attention, but probably to the oscillation of the neural processes of inhibition and facilitation. The oscillation of colors is thereby connected with other phenomena of vision.

Introduction

In conducting investigations on vision it has been the common practice to make the observations with a single eye, and to disregard completely the influence of the other eye which usually was kept in a state of darkness adaptation. In view of the complicated nature of the sense of vision, this procedure involves many uncertainties since there is no way of separating effects which are purely retinal from those which pertain to the nervous tracts and the cortical centres. An example of this uncertainty is found in the phenomenon of contrast, which has been attributed by Helmholtz to psychological action in the cortex, and by Hering and by Mrs. Ladd Franklin to the action of light on photochemical substances in the retina. Uniocular investigations have also suggested the idea that the production of white by complementary colors results from similar retinal reactions. Uniocular experimentation at best involves the action of one-half of the visual apparatus, whereas the mechanism of vision is so constituted that perfect action can be obtained only binocularly.

In binocular investigations the cortical actions, in part at least, can be separated from those which occur in the retina. The effects of color mixture can therefore be attributed to the visual centres in the cortex where color sensations are elaborated. Hence it is rather surprising to find that so little quantitative work has been done on binocular color mixtures, and on binocular phenomena generally.

From the experimental standpoint there is perhaps some reason for the paucity of measurements in this field. In binocular researches the spectroscopic apparatus must necessarily be much more elaborate than that needed for uniocular investigations. While binocular fusion of two color fields can

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be accomplished by suitable methods with great ease and with no perceptible strain upon the eyes, the observations involve color rivalries or oscillatory effects which at times render measurements difficult.

For a long time the possibility of binocular fusion of colors was a subject of much controversy (9, vol. 3, p. 505). Dove, Regnault, Brücke, Ludwig, Panum and Hering, among the earlier observers, strongly affirmed the occurrence of fusion, while Meyer, Volkmann, Meissner and Helmholtz were equally positive in asserting the opposite view. The existence of binocular fusion of colors has now ceased to be a matter of dispute, as numerous methods of observation with the aid of colored glasses, papers and metal disks, as well as the spectrum, have been devised to illustrate the phenomena. Schenck (11), Stirling (12), Dawson (4, 5), Hecht (8) and von Kries* have described qualitative methods for obtaining fusion, though von Kries confesses that only with great difficulty did he succeed in obtaining unmistakable results. Several of these observers found that the presence of contours or patterns greatly facilitated the observations. Foucault and Regnault (7), as well as Dove (6), obtained binocular fusion by simultaneously viewing through separate telescopes two colors of a spectrum projected upon a screen. Trendelenburg (13, 14), however, was the first to make accurate comparative measurements of uniocular and binocular fusion of complementary spectral colors to form white, and of red and green colors to produce yellow. The measurements of complementary colors were amply confirmed and extended by Rochat (10). Rochat, as well as Trendelenburg, noticed that the binocular fusion of complementary colors follows the same rules that govern uniocular fusion, but that the intensities of the colors of shorter wave-lengths are smaller for binocular than for uniocular mixtures in the less refrangible portion of the spectrum as far as the wave-length $480\text{ m}\mu$. The intensities of this color were the same for both types of fusion, but for still shorter wave-lengths the binocular intensities became the greater. The reason for this change at the wave-length $480\text{ m}\mu$ is not apparent. But it may be remarked that F. Allen (1) found this hue to be an equilibrium color which consequently does not influence inductively its complementary sensations.

These facts are clearly brought out in Tables I and II, which are reproduced from the papers of Rochat and Trendelenburg. The wave-lengths of the component complementary colors are within brackets, and the proportional intensities, originally measured in hundredths of a centimetre of slit width, are given by the numbers before the brackets. The column of "Proportion" on the right of the tables gives the ratios of the uniocular to the binocular intensities of the shorter wave-lengths. The ratio $3 : 2.2$, for example, is equivalent to $1.37 : 1$.

It is worthy of note, especially in Table II, that the intensities of the shorter wave-lengths from $498\text{ m}\mu$ to $491\text{ m}\mu$ are conspicuously greater than those of the longer and shorter wave-lengths $502\text{ m}\mu$ and $449\text{ m}\mu$. If the fundamental sensation curves of König and of Abney in Figs. 1 and 2 be

*See Ref. 9, Vol. 3, p. 529.

TABLE I

INTENSITY OF COMPLEMENTARY COLORS (ROCHAT)

Unocular	Binocular	Proportion
1 (671 $m\mu$) + 3 (493 $m\mu$) = White	= 1 (671 $m\mu$) + 2.2 (493 $m\mu$)	1.37 : 1
1 (656 $m\mu$) + 5.7 (492 $m\mu$) = White	= 1 (656 $m\mu$) + 4.3 (492 $m\mu$)	1.34 : 1
1 (617 $m\mu$) + 6 (489 $m\mu$) = White	= 1 (617 $m\mu$) + 5.4 (489 $m\mu$)	1.1 : 1
1 (585 $m\mu$) + 11.5 (485 $m\mu$) = White	= 1 (585 $m\mu$) + 11.8 (485 $m\mu$)	0.97 : 1

TABLE II

INTENSITY OF COMPLEMENTARY COLORS (TRENDELENBURG)

Unocular	Binocular	Proportion
1 (671 $m\mu$) + 5.5 (502 $m\mu$) = White	= 1 (671 $m\mu$) + 2.05 (502 $m\mu$)	2.7 : 1
1 (616 $m\mu$) + 23.7 (498 $m\mu$) = White	= 1 (616 $m\mu$) + 11.8 (498 $m\mu$)	2 : 1
1 (600 $m\mu$) + 22.8 (495 $m\mu$) = White	= 1 (600 $m\mu$) + 13.5 (495 $m\mu$)	1.7 : 1
1 (589 $m\mu$) + 17.1 (491 $m\mu$) = White	= 1 (589 $m\mu$) + 13.55 (491 $m\mu$)	1.3 : 1
1 (570 $m\mu$) + 8.7 (449 $m\mu$) = White	= 1 (570 $m\mu$) + 17.3 (449 $m\mu$)	0.5 : 1

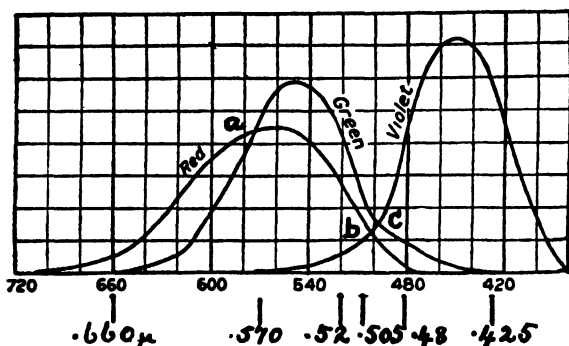


FIG. 1. Normal sensation curves (König). The six colors specially marked are equilibrium colors. (From Parsons, J. H. *Colour vision*, 2nd ed. Cambridge Univ. press, 1924, p. 235.)

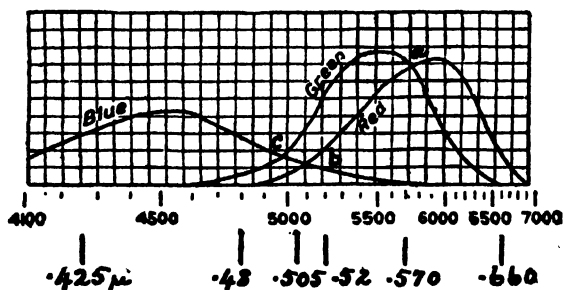


FIG. 2. Normal sensation curves (Abney and Watson). The six colors specially marked are equilibrium colors. (From Parsons, J. H. *Colour vision*, 2nd ed. Cambridge Univ. press, 1924, p. 260.)

examined, it will be seen that the group of wave-lengths from 498 $m\mu$ to 491 $m\mu$ fall at the lowest parts of the curves, whereas their complementary colors, 616 $m\mu$ to 589 $m\mu$, correspond to the much greater ordinates of the red and green curves. As the sensation of white is due to equal stimulation of all three primary sensations, red, green and violet, it is evident that extra large intensities of the shorter wave-lengths in question will be required to balance the excitation of the longer wave-lengths. But when the short-wave components are greater than 498 $m\mu$ and less than 491 $m\mu$, their ordinates in the sensation curves increase in length, which indicates that the disparity between the intensities of

the complementary components becomes less than before, and therefore less intense stimulation by the shorter wave-lengths is required to balance the excitation by the longer.

This explanation applies to all the binocular intensities except the last value 17.3 in Table II, which, however, is the mean of only two measurements 13.6 and 21, the former of which is the same as for the wave-length 491 $m\mu$. The enhancing inductive action of stimulation of one eye upon the sensitivity of the other is probably sufficient to account for the smaller values of the binocular intensities than of the uniocular.

During the course of the present investigation, two sets of binocular complementary colors were obtained by the writer and W. A. Allen. The sets differ from each other and also from those obtained by Helmholtz for uniocular observation, but not by greater amounts than those which are generally found between estimates of different observers. In Table III the first and fourth columns contain the pairs of complementary colors according to Helmholtz (9, vol. 2, p. 126). The wave-lengths in the first column were used also by the writer and his assistant for observation with the left eye, while the second and third columns contain respectively the binocular complementaries obtained with the right eye by himself and by W. A. Allen.

TABLE III
COMPLEMENTARY COLORS

Helmholtz. Also used by J.F.A. and by W.A.A. for observation by left eye. Wave-length, $m\mu$	Right eye, J.F.A. Binocular Wave-length, $m\mu$	Right eye, W.A.A. Binocular Wave-length, $m\mu$	Helmholtz Uniocular Wave-length, $m\mu$
656.2	500.5		492.1
607.7	490		489.7
585.3	476.3		485.4
492.1	613	630	656.2
489.7	597	599.3	607.7
485.4	592	592.3	585.3
482.1	589.5	584.2	573.9
464.5	583.3	580.8	567.1
461.8	578	572	564.4

The Binocular Production of Yellow

In addition to his observations on complementary colors, Trendelenburg also measured the uniocular and binocular intensities of the red and green hues required to form the sensation of yellow, as in the Rayleigh equation, $671\ m\mu + 535\ m\mu = 589\ m\mu$. It was also found that the uniocular intensity of the shorter wave-length, green, was always greater than the binocular in the ratio of from 4 : 1 to 14 : 1, according to the observer.

F. Allen has suggested that two of the three fundamental color sensations correspond to the wave-lengths $687\text{ m}\mu$ (red) and $530\text{ m}\mu$ (green), for the reason that these colors together with the third primary, probably $410\text{ m}\mu$, elicited conspicuously large inductive effects of depression and enhancement of retinal sensitivity. The color $550\text{ m}\mu$, however, excited inductive effects nearly if not quite equal in magnitude to those of $530\text{ m}\mu$, so that it was uncertain as to which hue of green precisely was the fundamental. There seemed to be no way of deciding between them. It is evident, however, that these two fundamental colors should fuse binocularly into yellow. The mixture $687\text{ m}\mu$ and $530\text{ m}\mu$ was tried, but the fusion yellow was greenish in hue. When the wave-length $550\text{ m}\mu$ was substituted for $530\text{ m}\mu$, the fusion showed no tinge of the component colors and differed from spectral yellow only in saturation. This hue of green therefore probably represents very closely the fundamental green sensation. While the colors of the Rayleigh equation fuse equally well into yellow, yet the wave-length $671\text{ m}\mu$ is not as pure a red as $687\text{ m}\mu$, nor does it produce such pronounced inductive reactions. The latter wave-length is therefore to be preferred as the fundamental red color.

The production of a yellow sensation by the binocular fusion of red and green colors throws considerable light upon one of the most important questions of color vision, that is, whether yellow is a simple or a compound sensation. The uncertainty regarding this question has been one of the chief reasons for the numerous theories of color vision, many of which are based on the assumption that yellow is quite as unique a sensation as red and green. It has been known from the time of Newton that uniocular fusion of red and green colors gives a yellow sensation. This experiment has been considered to be indeterminate since the component colors might be regarded as uniting to form white, while the yellow color, assumed to be evoked in addition by both red and green, appeared of the same hue as spectral yellow but less saturated. When the fusion of red and green is made binocularly, however, no question of such retinal actions can possibly arise, and the formation of yellow must therefore be attributed to the fusion of a pair of primary sensations in the cortex itself.

By these exact and conclusive spectroscopic experiments of Trendelenburg, confirmed qualitatively by other investigators such as von Kries, and now by the writer, the yellow sensation has been unquestionably proved to be compounded of the primary sensations red and green. Theories of color vision which are based on the assumed primary character of the yellow sensation consequently become untenable.

Binocular Oscillation of Colors

The phenomena of binocular oscillation appear when different colors are simultaneously viewed by each eye, and they are characterized by the successive presentation in consciousness of one color or the other, with the color

resulting from their fusion occasionally intervening. When, for example, red and green are the colors observed, there are seen in succession red, occasionally yellow resulting from fusion, green, and so on repeatedly.

The phenomena of binocular rivalry, or oscillation as it is preferable to term it, were originally attributed by Helmholtz, with the subsequent concurrence of other investigators, to the shifting of attention of the observer, an explanation arising no doubt from his custom of referring many phenomena, such as those of simultaneous contrast, to psychological action, when no satisfactory physiological principles were known with which they could be related. While the writer was observing the binocular fusion of complementary colors, the periodic oscillation of several of the pairs was so pronounced that it was decided to make time measurements in order to determine what degree of regularity existed in the oscillations and so test this application of the theory of attention.

There happened to be in the laboratory of F. Allen two similar Hilger constant deviation spectrometers mounted upon narrow bases but with their collimators turned in opposite directions. The telescopes were placed nearly parallel to each other as in Fig. 3, but separated by the distance between the eyes of the observer, so that each eye independently viewed a different but similar spectrum. After a little practice with the accommodation of the eyes, the colors could easily be fused with no perceptible effort, and the periods of oscillation measured. Illumination was provided by two 400-watt lamps, one in front of each collimator slit. A third collimator was used to direct a beam of white light by reflection into the telescope for the left eye, where it appeared just above the color patches in the field of view. The white light was used for comparison during the observations on binocular complementary fusion. A single patch of white light, however, is of very little value. For if white light falls upon one eye while the other has only a dark field in front of it, the fusion of the two gives an unsatisfactory gray field which is useless for purposes of comparison. The intensity of each color was controlled by the width of the slit, and the sizes of the patches of light were regulated by shutters both at the slits and in the eyepieces.

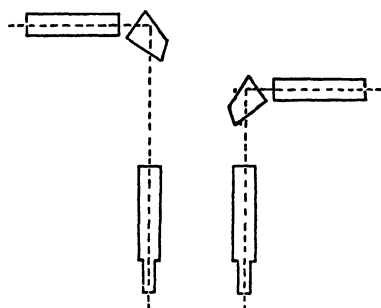


FIG. 3. *Arrangement of two spectrometers for observations of binocular fusion and oscillation of colors.*

For measuring the periods of oscillation a rotating drum chronograph was used, on which a timing pen, operated electromagnetically by a clock, marked off half-second intervals. Close beside the time recorder, a second pen was attached to an arm about 20 cm. long which was pivoted in the middle and adjusted so that its excursions ranged about a centimetre on each side of the time tracing. This pen was manipulated by the observer. While watching the binocular oscillation of colors, the observer swung the pen

above or below the line when the patch of light changed from one color to the other. Two such graphs are shown in Figs. 4 and 5. In all investigations on the senses, a period of training is always necessary in order to secure reliable and consistent measurements of any kind. Therefore in recording the periods of color oscillation considerable practice is necessary. The great difficulty in the present research lay in inducing the hand to shift the tracing pen at the instant a change of color occurred. No warning is given to prepare the mind for the change. One simply becomes aware that a different color is in view. In spite of this difficulty, quite consistent results were finally obtained for several pairs of colors as well as for a single pair at different intensities.

A third pen, set immediately above the tracing pen, was sometimes found useful. It was operated electromagnetically by an assistant whose duty it was to watch the eyes of the observer and record on the drum the moments of winking. In this way the connection of winking with the rate and nature of the oscillation was ascertained.

Results of Observations

In Fig. 4 is shown a typical chronographic record of the binocular oscillation of the colors red, $687\text{ m}\mu$, and blue, $480\text{ m}\mu$. The graph reads from left to right. The horizontal line of dots, A, records the time, the distances between successive dots representing half-second intervals. The

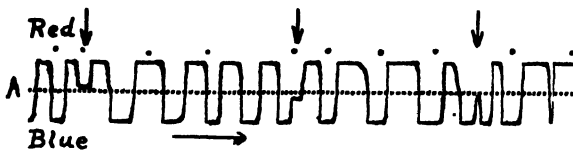


FIG. 4. Graph showing periodic binocular oscillation and fusion of red and blue colors. Horizontal line of dots, A, indicates half-second intervals. Upper line of dots indicates moments of winking. Vertical arrows indicate fusion of colors.

full line represents the observed oscillations from one color to the other, the parts above the horizontal time line representing the red phases, and the parts below the horizontal, the blue phases of the oscillations.

The three places where the full line breaks at the time line represent intervals of fusion. The dots above the oscillating line represent moments when winking occurred. There is evidence from this graph that fusion of colors is generally only an occasional episode in the more regular oscillations.

Fairly regular winking is a natural process to which the eyes are accustomed and to which the actions concerned in vision are adapted. It is therefore to be expected that when winking is inhibited the effort required for that purpose will influence visual processes in some degree. The graph shown in Fig 5, which was obtained with the same colors and under the same conditions

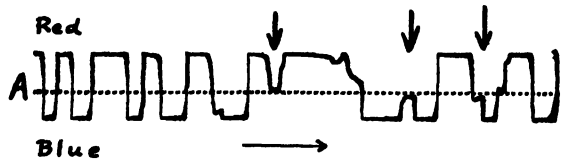


FIG. 5. Graph showing periodic binocular oscillation and fusion of red and blue colors. Horizontal line of dots, A, indicates half-second intervals. Vertical arrows indicate fusion of colors.

as for Fig. 4, except for the absence of winking, shows much greater irregularity than the previous figure. The irregularity increases with the time during which winking is restrained.

In Tables IV and V are shown the periods of time during which the red color, $687\text{ m}\mu$, in the right eye, and the blue color, $480\text{ m}\mu$, in the left eye, were visible, as well as the occasional periods during which fusion occurred. Both sets of times were obtained by the writer with colors of fairly high intensities. No figure corresponding to Table IV is given, but the data in Table V are obtained from the graph in Fig. 5. These measurements are

TABLE IV
PERIODS OF OSCILLATION AND OF FUSION (J.F.A.). WINKING

Red, $687\text{ m}\mu$ in right eye, sec.	1.50	1.00	1.25	1.50	0.75	1.25	1.75	1.25	2.00	1.50	2.00	2.25	1.25	1.25	1.25	1.50	Av.
Blue, $480\text{ m}\mu$ in left eye, sec.	1.50	2.00	2.25	2.00	1.00	1.50	1.50	2.50	1.00	1.50	1.50	1.25	3.00				1.73
Fusion, sec.	0.75	1.25	0.75	1.25	1.00	0.25	3.00	0.50	2.50								1.25

TABLE V
PERIODS OF OSCILLATION AND OF FUSION (J.F.A.). NON-WINKING (SEE FIG. 5)

Red, $687\text{ m}\mu$ in right eye, sec.	1.25	1.25	2.50	1.25	2.00	1.35	4.50	2.50	2.00	Av.
Blue, $480\text{ m}\mu$ in left eye, sec.	1.25	1.50	1.00	1.75	2.00	3.00	1.50	1.00	2.00	1.66
Fusion, sec.	0.75	0.75	1.25	1.00	0.75	0.50				0.83

illustrative of the variation in magnitude of consecutive periods. Since in the absence of precise knowledge of what is happening in the eyes, the individual periods are of little importance, in Table VI only average periods are given.

By comparing the values for the two observers in Table VI it will be noticed that quite generally the periodic times for the red and blue colors are reversed. Where W.A.A. finds the periods for red longer than for the blue, J.F.A. finds the opposite to hold and the periods for blue are longer than for red. In the case of both observers, however, when the colors in the eyes were interchanged, the periodic times of oscillation were also reversed.

It was formerly noticed by F. Allen (2) that in a few minutes after any sense organ had been stimulated, it acquired an enhanced state of excitability for subsequent stimulation. In Section 2 of Table VI, repetition of oscillation measurements after a rest of ten minutes showed diminished periods for both red and blue phases. These reduced times may therefore be attributed to the enhanced activity of the receptors. While the periods of oscillations were shorter, the fusion periods, in the case of W.A.A., were doubled.

TABLE VI

PERIODS OF OSCILLATION AND FUSION FOR W.A.A. AND J.F.A.†

1. Right eye, red, 687 $m\mu$; left eye, blue, 480 $m\mu$

	Both colors high intensity	Same, non-winking	Both colors low intensity	Intensities equalized	Red, low intensity; blue, high intensity	Red, high intensity; blue, low intensity
W.A.A.						
Mean period for 687 $m\mu$, sec.	1.57	2.00	1.69	2.17	1.61	1.89
Mean period for 480 $m\mu$, sec.	1.68	1.92	1.58	2.08	2.11	1.65
Mean period for fusion, sec.	1.08	1.50	0.87	1.33	1.29	1.16
J.F.A.						
Mean period for 687 $m\mu$, sec.		*1.56	1.59	1.38	1.15	1.50
Mean period for 480 $m\mu$, sec.		*2.25	1.70	1.77	1.73	1.46
Mean period for fusion, sec.		*1.50	None	1.13	1.04	1.15

2. Colors interchanged. Right eye, blue, 480 $m\mu$; left, red, 687 $m\mu$

	Low intensities		Repeated after 10 min. rest	
	W.A.A.	J.F.A.	W.A.A.	J.F.A.
Mean period for 687 $m\mu$, sec.	1.62	1.77	1.20	1.57
Mean period for 480 $m\mu$, sec.	1.79	1.58	1.56	1.40
Mean period for fusion, sec.	0.65	None	1.25	None

3. Other pairs of colors. Right eye, red, 687 $m\mu$; left eye, blue, 450 $m\mu$, and green, 550 $m\mu$. Intensities equalized

	W.A.A.		J.F.A.	
Mean period for 687 $m\mu$, sec.	1.88	0.97	1.43	1.13
Mean period for 450 $m\mu$, sec.	1.69		1.86	
Mean period for 550 $m\mu$, sec.		2.18		2.00
Mean period for fusion, sec.	1.36	1.25	1.03	1.00

† All observations were made with winking at normal rate, except those marked "non-winking".

* In these measurements for J.F.A. the red was of low intensity and the blue of high intensity

Both observers also found that when green, 550 $m\mu$, was one of the oscillating colors, its influence was always predominant. This fact is given numerical verification in Section 3, Table VI, where the green phase lasted a noticeably longer time than the red though the intensities of the colors were equalized.

It was discovered by Professor F. Allen (1) that six colors of the spectrum, 660 $m\mu$, 572 $m\mu$, 520 $m\mu$, 505 $m\mu$, 480 $m\mu$ and 425 $m\mu$, were incapable of either enhancing or depressing the sensitivity of the visual receptors. For this reason they were called equilibrium colors. Their peculiar character

was first noticed when they were used at high intensities. Whether in all cases they retain their equilibrium character at lower intensities as well, is open to question. From experimental evidence, however, it is quite certain that the color 505 $m\mu$ does possess an equilibrium character from the lowest to the highest range of intensities; and the same is probably true for some wave-length close to 570 $m\mu$ or 572 $m\mu$. For theoretical reasons which will shortly be discussed it seemed advisable to examine pairs of these peculiar colors for their powers of oscillation. For since they have an equilibrium nature they should not oscillate with each other at all. The results of this examination are given in Table VII. The second and third pairs of colors are not equilibrium colors. Where no oscillation occurred steady fusion of colors took place.

TABLE VII
BINOCULAR OSCILLATION AND FUSION OF COLORS

Pairs of colors		Oscillation at low intensity	Oscillation at high intensity	Pairs of colors		Oscillation at low intensity	Oscillation at high intensity
$m\mu$	$m\mu$			$m\mu$	$m\mu$		
570	660	Slight	Slight	480	570	Slight	Some
535	687	Decided	Decided	480	520	None	None
535	671	Decided	Decided	425	660	Some	Some
520	660	Some	Some	425	570	Slight	None
505	671	Some	Some	425	520	None	None
505	570	None	None	425	505	None	None
480	660	Some	Some	425	480	None	None

From Table VII it will be noticed that the equilibrium colors, 425 $m\mu$, 480 $m\mu$, 505 $m\mu$, and 520 $m\mu$, in any combinations that were tried, gave no indication of binocular oscillation. Fusion of the two colors was always maintained for any length of time of observation. When 425 $m\mu$ was used with 570 $m\mu$, slight oscillations were observed at low intensities, but complete and stable fusion occurred at high intensities. Special attention may be directed to the fact that the combination 505 $m\mu$ and 570 $m\mu$ gave not the slightest indication of oscillation, but perfect fusion was maintained at all intensities. The color 480 $m\mu$ with 570 $m\mu$ was found to give at low intensities slight evidence of oscillation which was more pronounced when the intensities were much increased. The combination 570 $m\mu$ and 660 $m\mu$ gave only slight oscillations at all intensities. The equilibrium band of color at 660 $m\mu$ is so narrow that it requires a two-prism spectrometer to disperse the spectrum sufficiently to obtain it. In these experiments single prisms were used so that the color in question could not be obtained sufficiently pure to be free from oscillatory effects. Probably a more complete study of these oscillatory phenomena would enable the exact equilibrium hues to be determined.

Theoretical Considerations

The usual name given to the phenomena of oscillation is color rivalry. Many peculiarities of its operation have been observed and descriptions of them may be found in the treatises on color vision. Obviously the term "rivalry" itself affords no suggestion of an explanation of the alternately successful competition of the colors for predominance in consciousness. Taken by itself the term "oscillation" is open to the same objection unless some condition can be found which does the oscillating of which the color observed is merely the index.

As mentioned above, Helmholtz ascribed the phenomena to fluctuations of attention. The periodic nature of the observations which have been described, the reversal of periodic times with the interchange of colors in the eyes, and the change of periodic times with intensities, are all opposed to that theory. But the theory of attention as an explanation of rivalry is rendered untenable by the fact that many combinations of equilibrium colors fail completely to give any oscillatory effects. This is particularly true of the colors, yellow 570 $m\mu$, and green 505 $m\mu$, which are the most complete equilibrium colors known. They are very dissimilar in appearance and the first is the brightest color in the spectrum. In addition to this evidence against the theory of attention, the writer found all attempts to hasten or delay the oscillations of colors by arbitrary fixation of attention to be futile; they were beyond voluntary observation or control.

Two neural processes, inhibition and facilitation, are well known in physiology, and these have been found by F. Allen (3) to be associated with all visual and indeed with all sensory activities. Their combined function is to control the sensitivity of the receptor apparatus, inhibition to depress and facilitation to enhance it. Every color is a physiological stimulus with individual characteristics, especially in regard to its power of eliciting these two inductive or sensory reflex processes. When the stimulating power of the color results in a perfect balance between them, the color has an equilibrium character. It is quite possible for a color to have this power at high intensities and not at low, or to possess it at low but not at high intensities of stimulation. When the color falls upon the retina of one eye the inductive processes, whether unbalanced or in equilibrium, control the sensitivity of the other retina. If when two colors fall upon the two eyes, the inductive processes exactly balance each other, they may be continually fused into a stable compound color with neither component able to predominate over the other. But when the inductive actions are unbalanced, as generally happens, the inhibitory process will then depress the reception of one color and allow the other to appear exclusively in consciousness, until the inductive actions become reversed and the first color in its turn becomes visible. Occasionally the inductive actions may balance each other for a second or two with the result that fusion of the two colors occurs, after which the oscillation again becomes evident.

If, when fusion of the two colors occurs, the three fundamental sensations at the same time are equally stimulated, the compound or fusion sensation is necessarily white. The binocular fusion of pairs of complementary colors is therefore precisely the same in nature as fusion in general, except that it always conforms to the special condition of equality of stimulation of all three sensations. When, however, equality of binocular stimulation is confined to the two fundamental sensations, red and green, the fusion product is not white but yellow.

The phenomena of binocular oscillation and fusion of colors in general, of binocular fusion of complementary and of equilibrium colors, and of the binocular production of yellow, are therefore indicated to be but different manifestations of the inductive neural processes inseparably associated with the action of light of different wave-lengths upon the eyes, combined with the equal or unequal stimulation of the three primary sensations red, green and violet, or with equal stimulation of only the red and green sensations.

Acknowledgment

The author desires to express his thanks to Professor Frank Allen, under whose direction the investigation was undertaken, for supplying the necessary experimental facilities.

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THE VISCOSITIES OF ACETALDEHYDE AND PARALDEHYDE¹

BY W. H. HATCHER² AND C. T. MASON³

Abstract

The viscosities of acetaldehyde, of paraldehyde and of mixtures of these are given at 15° C., using materials freshly distilled out of contact with oxygen.

Introduction

The necessity for viscosity data on acetaldehyde, paraldehyde and mixtures of these two required the determination of such values. This involved the preparation of these aldehydes and their use out of contact with oxygen. Results previously obtained (1) provided specific gravity data for these aldehydes and their mixtures. Also some unpublished work indicated that contamination of acetaldehyde with oxygen is significant only in the gaseous phase.

Preparation of Reagents

A high grade of paraldehyde was distilled fresh every day, and the fraction between 123° and 124° C. used. The same quality of paraldehyde was used with sulphuric acid for distillation of acetaldehyde, and this was redistilled, both processes being carried on in an atmosphere of nitrogen. The acetaldehyde was also freshly distilled each day.

Apparatus

After several trials an apparatus (Fig. 1) was devised on the principle of the Ostwald viscosimeter. It is roughly triangular in shape, having at *A* a side arm attached at right angles to the plane shown; through this side arm were admitted the liquids studied. Particularly, however, this arm served as a pivot about which the body of the viscosimeter could be rotated within the limits of the thermostat. *G* represents the side of the thermostat; *E* and *F* rests against which the vertical flow arm was held during an experiment. After filling at *A*, partial rotation filled this flow-arm to the same height for successive measurements, so that passage of the liquid past *B* and through *C* would be uniform. Thus the

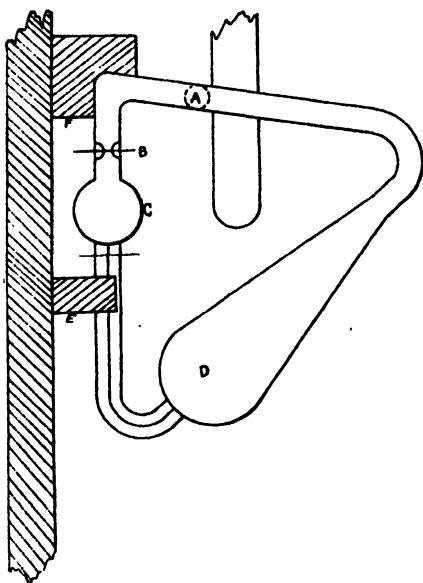


FIG. 1. Diagram of viscosimeter.

apparatus with the appropriate volume of liquid stoppered within could be kept in the same condition for each reading without opening.

¹ Manuscript received February 17, 1933.

² Contribution from the Department of Chemistry, McGill University, Montreal, Canada.

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Experimental

Calibration of the viscosimeter was effected with boiled, distilled water. Where mixtures of the aldehydes were used, mixing was previously carried out by weighing, the appropriate volume being later pipetted in. These operations which involved aldehydes were carried out in an atmosphere of pure nitrogen. Although some contact with air was at times unavoidable, yet no aldehyde vapor which had come into contact with oxygen was able to get into the viscosimeter. Extreme precautions as to cleanliness were observed throughout. The temperature maintained throughout was 15° C., the bath variation being $\pm 0.1^\circ$ C. at the most.

Results

The results which follow were obtained using a constant liquid volume of 20 cc. at 15° C., and are the mean of times of flow which agreed to within 0.5 sec. Calibration with water showed a time of flow of 338.2 sec.

The results for the pure aldehydes and their mixtures are included in Table I. For purposes of comparison the composition of the mixtures (by weight) was plotted on a large scale against time of flow; from this the times of flow were read off at 10% intervals. Using these values and the specific gravities at these percentages from the curve of Hatcher and Kay (1), the specific viscosities compared with water at 15° C. were calculated.

If the values shown in Table I be represented graphically a smooth curve is obtained lying (for the mixtures) always below the ideal joining acetaldehyde and paraldehyde, the maximum deviation occurring at 50% concentrations, where the actual specific viscosity is 63% of the ideal.

TABLE I
VISCOSITIES OF ACETALDEHYDE-PARALDEHYDE MIXTURES

Percentage acetaldehyde	Sp. gr.	Time of flow, sec.	Specific viscosity at 15° C.	Percentage acetaldehyde	Sp. gr.	Time of flow, sec.	Specific viscosity at 15° C.
0.0	0.9963	403	1.188	60.0	0.8650	141	0.3610
10.0	.9750	290	0.8368	70.0	.8448	126	0.3150
20.0	.9515	238	0.6702	80.0	.8250	113	0.2759
30.0	.9295	203	0.5584	90.0	.8057	101	0.2408
40.0	.9080	178	0.4783	100.0	.7865	92.3	0.2147
50.0	.8862	158	0.4144				

Using the value 0.1144 as the absolute viscosity of water (2, p. 10), the latter for paraldehyde becomes 0.1359 and for acetaldehyde 0.02456. This last compares with 0.02321 as found by Thorpe and Rodger (3). No value for paraldehyde was known previously.

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THE ALKALOIDS OF FUMARACEOUS PLANTS

V. THE CONSTITUTION OF ADLUMINE¹

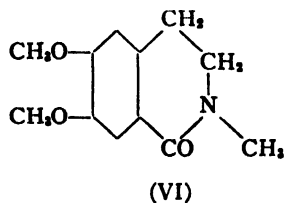
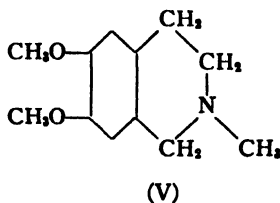
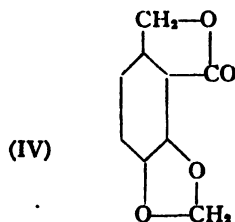
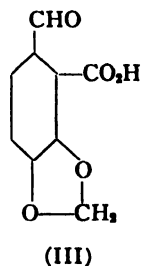
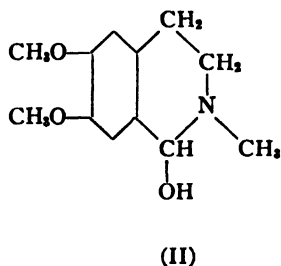
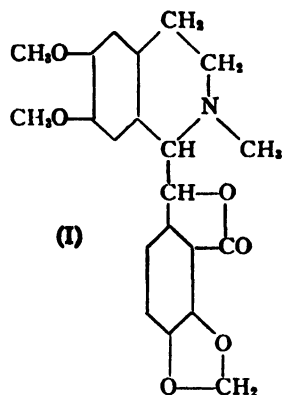
BY RICHARD H. F. MANSKE²

Abstract

A chemical examination of the alkaloid *adlumine*, which the author recently isolated from *Adlumia fungosa*, has disclosed an intimate relation to hydrastine and bicuculline. It differs from hydrastine only by the fact that the positions of the methylene-dioxy and the dimethoxy groups are reversed. Hydrolytic oxidation yields the expected products.

During an investigation dealing with the alkaloids of *Adlumia fungosa* (2) the author isolated an alkaloid having the empirical formula $C_{21}H_{21}O_6N$, to which the name *adlumine* was given. A methoxyl determination disclosed the presence of two such groups and the nitrogen appeared to be tertiary.

Since the alkaloid is isomeric with hydrastine a close relationship was suspected and this was further confirmed by the observation that oxidation with manganese dioxide in dilute sulphuric acid yielded a bluish fluorescent solution. If it be assumed that the nuclear substituent oxygen atoms occupy the same positions in this alkaloid as they do in hydrastine and in bicuculline the only possible formula for adlumine is (I).



¹ Manuscript received March 3, 1933.

Contribution from the National Research Laboratories, Ottawa, Canada.

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On this basis hydrolytic oxidation should yield 4, 5-dimethoxy-2 (β -methyl-aminoethyl)-benzaldehyde (II) and 2-carboxy-3, 4-methylenedioxy-benzaldehyde (III). Experimentally this supposition was readily verified. The aminoaldehyde (II) was first prepared from laudanosine by Pyman (3) and one is grateful to this author not only for the thoroughness with which it was characterized but also for the fact that all melting points were corrected. In the present case the free base (m.p., 123-124° C.)* was characterized by conversion to the picrate (m.p., 170° C.), and more specifically by the production from it, by treatment with alkali (Cannizzaro), of a mixture of 1, 2, 3, 4-tetrahydro-2-methyl-6, 7-dimethoxy-isoquinoline (V) (m.p., 84° C.) and the 1-keto-derivative (VI) (m.p., 126° C.). In all cases the properties of the products were those recorded by Pyman.

The acidic fragment was not isolated as such, but reduced at once with sodium amalgam and it then yielded 3, 4-methylenedioxy-phthalide (IV) which proved to be identical with a specimen similarly prepared from bicuculline (2).

Adlumine is thus the third representative of four possible alkaloids of closely related structure with the nuclear oxygen atoms in the same position. The existence of the final member in nature, namely, a tetramethoxy compound, is therefore highly probable, since it is evident that the phytochemical processes are capable of sufficient variation, because of the fact that methoxyl groups have been shown to occur in the two pairs of positions.

Experimental

Hydrolytic Oxidation of Adlumine—

Isolation of the Amino-aldehyde (II)

One gram of the alkaloid was dissolved in a mixture of 2 cc. of concentrated nitric acid and 8 cc. of water and heated on a steam bath for 20 min. The mixture was thoroughly cooled, rendered strongly alkaline with 50% aqueous potassium hydroxide and the liberated base extracted with several successive portions of ether. The combined extract was dried over sodium sulphate, the greater part of the solvent removed and the residual solution cooled. The crystalline base was recrystallized once from ether and melted at 123-124° C.

The *picrate* was recrystallized from methanol by the cautious addition of ether, and melted sharply at 170° C.

The total amount of base obtained from two grams of the alkaloid was heated for 30 min. on a steam bath with an excess of methanolic potassium hydroxide and the tetrahydro-isoquinoline (V) and its 1-keto-derivative (VI) separated and purified by Pyman's method. The former was obtained in fine needles melting at 84° C. and the latter in the form of large flat plates melting at 126° C.

* All melting points are corrected.

*Isolation of 2-Carboxy-3:4-methylenedioxy-benzaldehyde
and its Conversion to the Phthalide (IV)*

The alkaline solution from which the aminoaldehyde (II) had been extracted with ether was acidified with sulphuric acid and exhausted with ether. The combined extracts were washed with several small successive portions of water and most of the ether distilled on a steam bath. The remaining solvent was removed *in vacuo* and the solid crystalline residue reduced with sodium amalgam in dilute sulphuric acid solution. The aqueous solution was then extracted with ether, the solvent removed, and the residue recrystallized from hot water with the aid of charcoal. As thus obtained the 3, 4-methylenedioxyphthalide (IV) consisted of colorless elongated needles which melted alone or admixed with a specimen obtained from bicuculline at 232° C.

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THE ALKALOIDS OF FUMARACEOUS PLANTS

VI. *Corydalis sempervirens* (L.) PERS.¹

BY RICHARD H. F. MANSKE²

Abstract

Corydalis sempervirens has yielded six alkaloids, two of which, bicuculline and bicucine, had been previously found in *Dicentra cucullaria*. The similarity of these two plants is further demonstrated by the presence in the former of cryptopine and, as in the case of other fumaraceous plants, protopine. A new alkaloid, $C_{19}H_{17}O_6N(?)$, which has been named *capnoidine* appears to be isomeric with adlumidine but is not identical with it. A very small amount of an unidentified base melting at $201^\circ C.$ was also obtained.

Although a number of Asiatic species of *Corydalis* have been very thoroughly investigated in regard to the alkaloids present, this is not true of species native to America. The only record of such an investigation is a cursory one by Heyl (1) dealing with *C. aurea*, Willd.

In view of the fact that, as recorded in earlier papers of this series, a number of new alkaloids have been isolated from American species of *Dicentra* it seemed particularly desirable to examine some American *Corydalis* species, and the present communication deals with *C. sempervirens* (L.) Pers. (*C. glauca*, Pursh.). Some difficulty was experienced in obtaining sufficient material for this investigation, so that the record is to be regarded as not quite complete and it is proposed to continue the investigation when more material becomes available.

The total quantity of alkaloid found in this plant was exceptionally small, but this is probably due to the fact that it had reached an advanced state of maturity at the time of collection. Nevertheless, six alkaloids have been isolated in a state of purity. Protopine, which seems to be a constant constituent of all fumaraceous plants, was found in quantity. Two of the other alkaloids were identical with the bases (α and β) previously isolated from *Dicentra cucullaria* (2). Alkaloid (α), which has proved to be new and whose constitution has been elucidated, has recently been named *bicuculline* (3). Alkaloid (β) has now been more completely characterized and since it also appears to be new, the name *bicucine* is proposed for it. Some difficulty has been experienced in obtaining satisfactory carbon analyses, but it is highly probable that the formula $C_{20}H_{19}O_7N$ is the correct one. In this eventuality it may bear the same relation to bicuculline that nor-narceine bears to narcotine.

A fourth alkaloid, which also may belong to this class of bases, was obtained in minute amounts. Analyses indicate the formula $C_{19}H_{17}O_6N$ (or $C_{19}H_{15}O_6N$). This is isomeric with the alkaloid adlumidine which the author has isolated from *Adlumia fungosa* (4). Furthermore, both alkaloids melt at 235° , but when admixed the melting point is depressed to 210° .

¹ Manuscript received March 4, 1933.

Contribution from the National Research Laboratories, Ottawa, Canada.

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* Melting points are corrected.

sintering taking place at 206° C. It is proposed to coin the name *capnoidine* for this base, the word being derived from the term Capnoides, which has been used by some botanists for the genus *Corydalis*.

It may be of interest to call attention to the close relation which the alkaloids of *C. sempervirens* bear to those of *D. cucullaria*. Not only do protopine, bicuculline and bicucine occur in both plants, but the similarity extends to the presence in both species of cryptopine which until recently had been found only in opium. In this connection it is relevant to point out that the alkaloid previously isolated from *D. cucullaria* and regarded as cryptopine has been further identified as such by comparison with an authentic specimen from opium which was kindly supplied by Dr. R. D. Haworth of Armstrong College, Newcastle-upon-Tyne.

The sixth alkaloid was obtained in such minute amounts that there was insufficient even for a microanalysis. It melts sharply at 201° C. and has the appearance of homogeneity. It is proposed to refer to this substance as alkaloid γ until further characterization is possible.

Experimental

The procedure outlined in some detail (4) in a communication dealing with *Adlumia fungosa* has been strictly followed. There was available a total of 3850 gm. of dried plant material of which the woody tap roots constituted 325 gm. The quantity of total alkaloid from the latter was sufficient only for the isolation and purification of protopine and bicuculline, and the mother liquors from these were combined with the corresponding fractions from the aerial portion. In the following record the designations of the various fractions are the same as those previously given (4).

Isolation of Fumaric Acid

This acid was isolated in precisely the same manner as from *A. fungosa*. Comparison with an authentic specimen as well as with the acid from the above source proved its identity. After appropriate purification it melted and sublimed at 295° C. About 4 gm. was obtained from the roots and an equal amount from the stems and leaves.

Isolation of Capnoidine, C₁₉H₁₇O₆N(?)

The chloroform extract (C) was freed of solvent as far as possible on a steam bath and then evaporated several successive times with methanol. Crystallization of a sparingly soluble hydrochloride was complete after several days. The substance was filtered off, washed with cold methanol and recrystallized by the addition of methanol to a concentrated aqueous solution. After filtering and washing, the hydrochloride (m.p., 244° C.) as thus obtained was treated with excess potassium hydroxide in aqueous solution. The precipitate which rapidly crystallized was filtered off and thoroughly washed with water. It then melted at 233° C. Recrystallization was readily effected by adding hot methanol to a concentrated chloroform

solution of the base, and yielded brilliant stout prisms of *capnoidine* which melted sharply at 235° C. in spite of the development of a slight brown color at a temperature a few degrees lower. Calcd. for $C_{19}H_{15}O_6N$; C, 64.59; H, 4.26; N, 3.97%. Mol. wt., 353. Calcd. for $C_{19}H_{17}O_6N$; C, 64.23; H, 4.79; N, 3.94%. Found: C, 64.94, 65.14; H, 4.70, 4.69; N, 4.07, 3.95%. Mol. wt., 337, 338 (Rast). Methoxyl, negative.

An intimate mixture of capnoidine and adlumidine began to sinter at 206° and was completely liquid at 212° C.

Isolation of Bicuculline and Bicucine

The combined mother liquors from the capnoidine were freed of methanol, the residue treated with hot dilute hydrochloric acid, filtered from some resin and thoroughly exhausted with ether. The aqueous solution (ASR) on appropriate treatment yielded a small amount of a base (BC) which proved on recrystallization to consist largely of the sparingly soluble capnoidine together with a small amount of bicuculline which was isolated from the mother liquors.

The ether extract (EC) yielded a few crystals of protopine (melting point and mixed melting point, 211° C.). It may be pointed out that protopine like allocryptopine is not completely precipitated by means of fixed alkali and it has repeatedly been obtained in appreciable amounts by extracting the aqueous filtrate with ether.

The precipitate (BCE) obtained by carbonating the alkaline filtrate (CES) was dried in a desiccator and extracted with chloroform. The filtrate was evaporated to a small volume and treated with a little methanol. In the course of several days about 0.5 gm. of almost colorless stout prisms separated, which after one recrystallization from chloroform-methanol melted sharply at 177° C. It was observed for the first time in this case that the resolidified melt on reheating melted at 195° C. Admixture with a specimen of bicuculline melting at 177° C. from *D. cucullaria* caused no depression in melting point, and the hydrochlorides from both sources exhibited identical properties.

The fraction of (BCE) which remained insoluble in chloroform was suspended in concentrated ammonia solution and a stream of gaseous ammonia passed in until only an insignificant amount of slimy residue remained. The latter was removed with the aid of charcoal and the filtrate placed in an evacuated desiccator over concentrated sulphuric acid. This is the only procedure which in the writer's experience will yield well-defined crystals of bicucine. Crystallization was slow but complete and the stout brilliant prisms thus obtained melt sharply at 217° C. When admixed with a specimen of alkaloid (β) (2) melting at 215° C. obtained from *D. cucullaria* by carbon dioxide precipitation from an alkaline solution, the mixture melted at 215° C. The yield was 0.7 gm.

Since previous analyses had not yielded figures which admitted of an unequivocal interpretation, this specimen was analysed after drying in a high vacuum over phosphorus pentoxide for 48 hr. during which time it lost about 1% of its weight. The combustion figures on the dried sample are in fair

agreement with the monohydrate of $C_{30}H_{19}O_7N$. This representation is preferred to the empirical formula $C_{20}H_{21}O_5N$. Calcd. for $C_{30}H_{21}O_5N$; C, 59.55; H, 5.21; N, 3.47; NMe, 3.72%. Found: C, 60.29; H, 5.22; N, 3.58; NMe, 3.86%. (Means of duplicates). (Herzig-Meyer). Methoxyl, absent.

Isolation of Protopine

Almost the entire quantity of alkaloids in the chloroform extract (AC) was obtained in the non-phenolic fraction (BS). The other fractions consisted for the greater part of insignificant residues largely contaminated with non-basic resins. Much larger quantities of plant material are necessary to permit of the isolation of appreciable amounts of pure products from these fractions.

The dried mixture of bases (BS) was dissolved in chloroform and a turbidity removed with charcoal. The filtrate was evaporated somewhat, treated with an equal volume of methanol and the solution again boiled with charcoal. This twofold treatment with charcoal is one that has repeatedly demonstrated its merits. Solutions of alkaloids obtained from precipitates or from aqueous solutions by means of immiscible solvents invariably contain small amounts of inorganic matter which is effectively removed by filtering a chloroform solution with the aid of charcoal. This treatment does not eliminate colored impurities appreciably. It has been found however that the second treatment with charcoal in the presence of as much methanol as feasible is very effective in removing color.

The filtrate from the second charcoal treatment was slowly evaporated on a steam bath to a thin syrup, which rapidly deposited large almost colorless stout prisms of protopine when it was seeded. After one recrystallization from chloroform-methanol the protopine was obtained in brilliant colorless crystals which alone or admixed with specimens of pure protopine from a number of other fumaraceous plants melted at $211^{\circ}C$. The mother liquors were again treated with charcoal and yielded a further amount, the total quantity obtained being 2.8 gm.

Isolation of Cryptopine and Alkaloid (γ)

The mother liquors from which no more protopine could be crystallized were all combined, evaporated to a small volume and acidified with a slight excess of concentrated hydrobromic acid in methanol. A small amount of protopine hydrobromide crystallized out in the course of several days. The filtrate from this was freed of methanol, the bases regenerated, and extracted with ether. The solvent was removed from the extract and the residue dissolved in an excess of aqueous oxalic acid. In the course of several days a crop of sparingly soluble crystals separated. This product was filtered off, washed with a little water and the base liberated by means of an excess of potassium hydroxide. It was extracted with a large volume of ether and the residue from the extract recrystallized from hot methanol. Except for an insignificant amount in the mother liquor the entire product was obtained in fine colorless prisms melting sharply at $221^{\circ}C$. Admixture with a specimen of cryptopine from opium or with one from *D. cucullaria* did not depress this

melting point. Color reactions of the bases from the three sources carried out side by side failed to show any differences. The total yield of cryptopine was 0.05 gm.

The mother liquor from the crystallization of the cryptopine oxalate was basified with excess potassium hydroxide and the liberated base removed with ether. The residue from the ether extract crystallized completely in contact with a little methanol. The crystals consisted of short stout prisms and appeared to be homogeneous; m.p., 201° C. The yield was somewhat less than 0.01 gm. Until this base is available in sufficient quantity for adequate characterization it will be referred to as alkaloid (γ).

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REVIEWS AND NOTES

A METHOD FOR FINDING SMALL LEAKS IN A HIGH-VACUUM APPARATUS¹

By F. R. TERROUX² and W. H. WATSON³

It is well known that the usual methods for finding a leak in a high vacuum system are of little use when the rate of leak is of the order of 10^{-4} mm. per hour or less, and arises from invisible defects in a metal-to-glass seal. Anyone who has experienced such leaks will admire the restraint with which Dunoyer (2, p. 154) describes the situation; "La recherche..... constitue un problème toujours fastidieux, occasion de grandes pertes de temps et parfois même insoluble". Obviously it may be possible to test each seal separately in a system of small capacity before assembly, but in spite of this, leaks of this magnitude may develop after the seals have been joined to the complete vacuum system.

The method here described makes use of the principle that the rate of leak depends on the difference of pressure prevailing across the fault. It provides a very simple technique for reducing the pressure on the outside of a seal, such as a pinch, without damaging the lead-in wires. When the pressure on the outside of a faulty seal is reduced in this way, the rate of leak of the evacuated apparatus will be markedly diminished, and if this is the only defective seal the rate of leak will become negligible. Clearly this technique can be applied to each seal in turn until the defective one is identified, and

measures can be taken to repair or replace it.

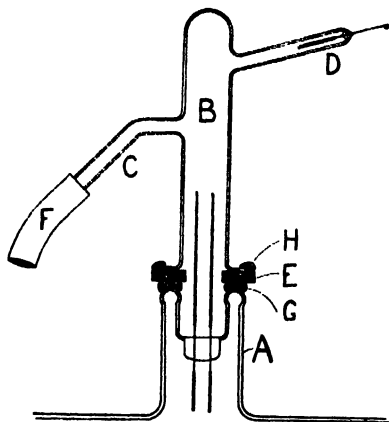


FIG. 1. Application of the method to a typical seal.

Reference to Fig. 1 will make clear the method actually used. The seal to be tested is shown at *A*. *B* is a tube of glass, or Pyrex, about 15 cm. long and 2 cm. in diameter with a flange about 1 cm. wide at the bottom. The side tube *C* serves to connect *B* to some form of vacuum pump. An electrode is sealed into the side tube *D* and the pressure in *B* can be estimated from the nature of the discharge when the electrode is connected to a small induction coil. The joint between the flanged end of *B* and the rim of seal *A* is made by using a rubber washer *E*, about 6 mm. thick,

pressed firmly against a layer of plasticene* *G* which has been worked smoothly

¹ Manuscript received February 24, 1933.

² Contribution from the Department of Physics, McGill University, Montreal, Canada.

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* Plasticene was used with great success by Cockcroft and Walton (1) in the construction of high voltage positive ray tubes.

into an annular shape before assembly. The joint is completed by pressing, with the fingers, another ring of plasticene *H* around the *outside* of the flange at the junction of *B* and *E*.

In practice, if the whole system is gently pressed together by hand and the pump started, then, provided the rubber connecting tube *F* is properly supported, the pressure in the space enclosed between the seal *A* and the tube *B* will rapidly be reduced to a few hundredths of a millimetre. This pressure is maintained by keeping the pump in operation. All that remains is to measure the rate of leak in the main vacuum system over an interval of a few hours. If the rate of leak is less than it was when the outside of *A* was open to the atmosphere, then seal *A* is the seat of the trouble. While this technique may be adapted to almost any conditions, the use of re-entrant seals in vacuum systems makes this method of detecting leaks very easy to apply.

In conclusion, the success of the above method suggests the idea that when a vacuum system is to be sealed off at very low pressure and is to retain its vacuum over a very long period, the use of double seals with the space between highly evacuated would give far greater protection at all metal-to-glass seals. Thus any ordinary seal which shows no appreciable leak in 24 hr. when exposed to atmospheric pressure, would, when backed by an evacuated space (and excluding accidents) show no appreciable leak over a period of many months.

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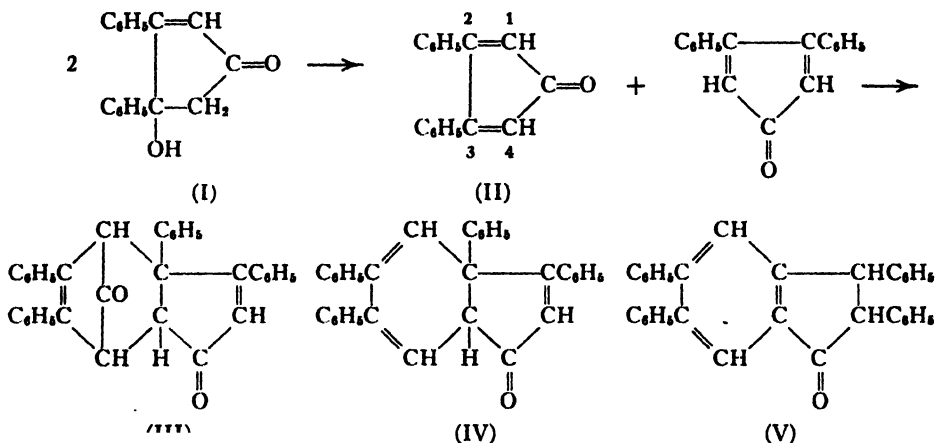
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THE STRUCTURE OF THE DIKETONE OBTAINED FROM ANHYDRACETONEBENZIL¹

BY C. F. H. ALLEN² AND E. W. SPANAGEL³

In a recent paper the writers (1) described the formation of a bimolecular product, $C_{24}H_{24}O_2$, from anhydracetonebenzil in various reactions. This substance had been made previously by Japp* who found that on being heated it lost carbon monoxide, with consequent formation of a new compound, $C_{22}H_{22}O$, that formed a phenylhydrazone. Japp did not suggest a structure for either of these compounds.

In the writers' previous paper (1, p. 4340) the assumption was made that the dehydration of anhydracetonebenzil (I) gave a cyclopentadienone (II), two molecules of which then reacted to produce the bimolecular product. Bearing in mind the addition reactions of Diels and Alder, it was assumed that to form the bimolecular product one molecule of the cyclopentadienone added to the ends of the conjugated system (1 : 4) in the second, yielding a tricyclic system containing a carbon monoxide bridge (III). On being heated



the carbon monoxide bridge could be split out, leaving a ketone (IV). Two isomeric ketones have been found, one of which gave a phenylhydrazone agreeing with Japp's; the second was formed by heating the first longer or to a higher temperature, and is a hydrindone (V).

The structure of the latter was carefully determined by a long series of degradation reactions that terminated in *o*-diphenylbenzene, showing that substance (III) must contain this skeleton.

The writers have also found that tetraphenylcyclopentadienone adds maleic anhydride to form a bicyclic system having a carbon monoxide bridge.

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1. ALLEN, C. F. H. and SPANAGEL, E. W. J. Am. Chem. Soc. 54 : 4338-4347. 1932.

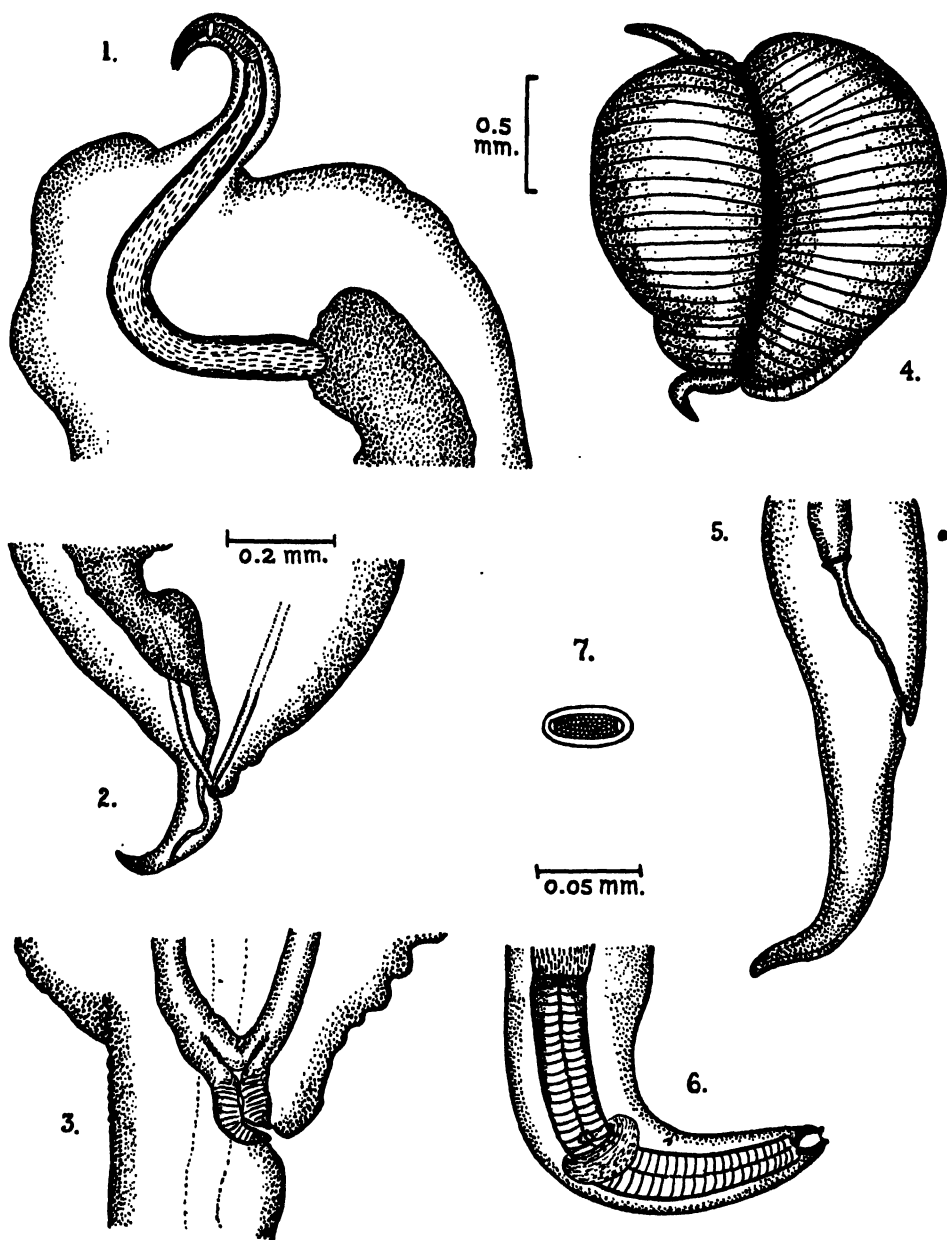
¹ Manuscript received March 21, 1933.

² Contribution from the Department of Chemistry, McGill University, Montreal, Canada

³ Assistant Professor of Chemistry, McGill University.

* Demonstrator in Chemistry, McGill University.

* References given in paper cited above.



FIGS. 1-7. *Tetrameres crami*, sp. nov. (1) Anterior part of female. (2) Posterior part. (3) Terminal portion of ovejector. (4) Female in toto. (5) Tail end of female, showing rectum. (6) Head end, dorsal view. (7) Egg, immature.

(To face page 336, *Canadian Journal of Research*, April 1933.)

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PENETRATION DURING SULPHITE COOKING¹

BY H. SAUNDERSON², H. W. JOHNSTON³ AND O. MAASS⁴

Abstract

This paper deals with some of the factors which influence the penetration of sulphite liquor into wood during the cooking process. The experimental technique for this purpose is described. The particular factors investigated in this paper are the influence of the calcium sulphite precipitate and the composition of the liquor on penetration.

Certain conclusions may be drawn from the experimental results presented. These are summarized as follows:

(1) The rate of penetration of sulphite liquor through a block of spruce wood is constant for a fixed low temperature. At a constant high temperature the rate of penetration increases continuously during the duration of a run, due to the opening up of the wood. This "cooking" action becomes evident at about 90° C. for heartwood, and below 70° C. for sapwood.

(2) The rate of penetration of sulphite liquor is greater for a higher concentration of free sulphur dioxide. This increase in rate with sulphur dioxide concentration becomes more noticeable at higher temperatures, indicating that at least a part of the effect is due to cooking action.

(3) The formation of a precipitate of calcium sulphite on the wood at high temperatures decreases the rate of forced penetration. This effect is more noticeable with porous wood, where the pressure differential is small. The plugging action is largely a surface effect, due to the formation of a layer of precipitate over the ends of the tracheids of the wood.

Introduction

The industrial importance of the sulphite process for manufacturing wood pulp has led to a detailed study of the various stages in the process. The greatest emphasis has been laid on the chemical reactions between the constituents of the wood and the solution of reagents—known technically as the cooking liquor—but recently an increasing amount of attention has been given to the penetration of the liquor into the wood, which obviously must precede any cooking action. The importance of a satisfactory preliminary penetration has been emphasized by Miller and Swanson (7, 8), Hügglund

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Contribution from Physical Chemistry Laboratory, McGill University, Montreal, Canada.

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⁴ Macdonald Professor of Physical Chemistry, Director of Research of the Physical Chemistry Division, McGill University, associated with the Forest Products Laboratories.

(4), Edwardes (2), and others, but little work has been done to study the way in which the sulphite liquor enters the wood, and the factors which influence this penetration.

Penetration is of two kinds, one caused by a difference in hydrostatic pressure on two sides of a piece of wood, resulting in a mass movement of liquid through the wood, which might be called forced penetration; and the other by a difference in the osmotic pressure of the liquor in two parts of a block of wood, resulting in a diffusion of ions or molecules gradually into the wood. This might be called natural penetration. The study of forced penetration would give information about the structure of the wood and its permeability to liquid flow under a differential hydrostatic pressure, while the study of natural penetration would give information about surface effects, and ionic and molecular diffusion. Forced penetration only will be discussed in this paper, the study of natural penetration and the practical implications of the work being dealt with later.

Johnston and Maass (5) studied the rate of penetration of liquids into jack pine using a measured pressure differential, varying the temperature, pressure, type of section, and concentration and nature of liquid. They found in general that the rate of flow varied almost directly with the differential pressure applied, and that it was much greater at higher temperatures. Tangential and radial sections showed very small penetrability in comparison with transverse sections.

Stamm (10, 11) has made a careful survey of the capillary structure of softwoods, using a number of different methods of investigation; electrical conductivity, liquid flow under differential pressure, and surface tension. From his work, he concluded that the flow of liquid in wood was through the tracheids and bordered pits, the size of the apertures in the latter being the limiting factor in the rate of flow. The mean capillary radius of the apertures in slash pine heartwood approximated 8×10^{-6} cm. and in sapwood 10×10^{-4} cm.

An investigation by J. W. Sutherland and O. Maass (12) concurrent with that reported in this paper, on the forced penetration of water and various solutions into spruce, rendered it unnecessary to study more than those factors in which the sulphite liquor might have an effect different from that of water. These were chiefly the cooking action of the solutions, the effect of varying concentrations, and the effect of the precipitation of a lime salt on or in the wood. The influence of temperature, and of pressure differential, and the difference between heartwood and sapwood were found to be the same with sulphite liquor as Sutherland and Maass found using water, so the study of these factors was very brief.

Apparatus

The problem in the construction of the apparatus was to devise a means of forcing liquid through disks of wood, maintaining known different pressures on the two sides of the disk, and such that the liquid above and below the

disk could be removed separately for analysis. It was necessary to make all the parts in contact with the liquid of material which would not be attacked chemically, and they had to be capable of standing a pressure of ten atmospheres.

The diagram of the apparatus constructed (Fig. 1) shows the connection of the various parts, and reference will be made only to those sections where the purpose or construction is not clear from the diagram.

The connections for the various parts were chiefly of $\frac{1}{8}$ -in. copper tubing, which combined mechanical strength, small volume of contents, flexibility, and chemical inertness. Valves marked on the diagram at (5) were regular brass water-gauge valves. The other valves shown in the diagram were also of brass, Crane $\frac{1}{8}$ -in. needle valves being used chiefly. It was found necessary to repack all the valve stem glands with asbestos and graphite to prevent slow leakage. Tees and crosses used to connect the different copper tubes were made of brass, the tubing being fastened to them by compression fittings. It was found by experience that a $\frac{1}{8}$ -in. tube fastened by a compression fitting did not leak if it were attached carefully, but in the few cases where $\frac{1}{4}$ -in. copper tubing was used for a connection, it was necessary to solder it into its fitting to make it tight.

The apparatus may be described most clearly by considering it as four main parts: (a) the clamp for holding the wood during a run; (b) the front-pressure system, which contains and controls the liquid to be forced through the wood; (c) the back-pressure system, which contains and controls the liquid which has been forced through the wood; and (d) the constant temperature bath.

(a) The Clamp

The centre of the apparatus was the clamp to hold the wood disk during a run. The location of the clamp is shown on Fig. 1 as (8) and the form of the clamp used shown in detail as Fig. 2. All the machined parts in contact with the solution were of Allegheny metal. The connection to the rest of the

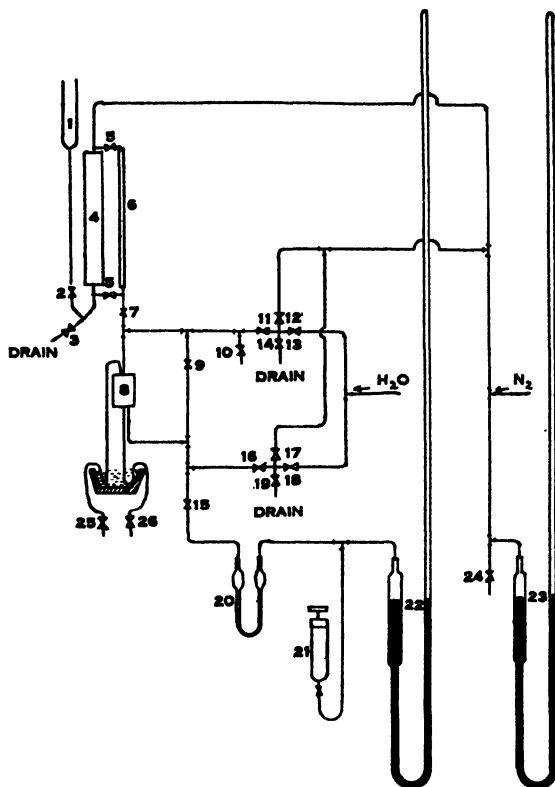


FIG. 1. Connections of penetration apparatus.

apparatus was of $\frac{1}{8}$ -in. copper tubing "sweated" into a tight fit with a high melting solder. In this clamp, the wood disk was surrounded by a ring having on its upper end a flange inward which was sharply undercut. The wood,

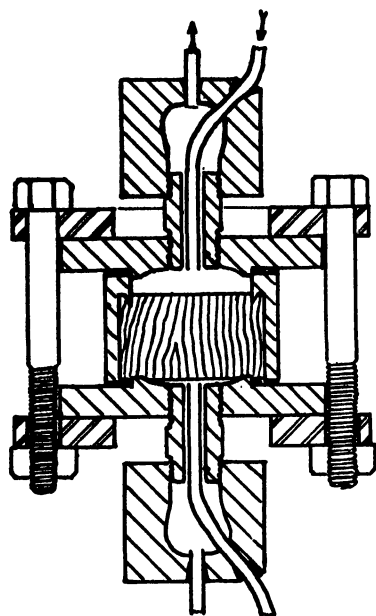


FIG. 2. The clamp for holding wood.

tube (6). The reservoir was of 2-in. brass tubing, 24 in. long, closed at both ends by brass caps. These caps were tapped to receive $\frac{1}{8}$ -in. close nipples which connected to $1\frac{1}{2}$ -in. brass tees, the side arm of each tee acting as socket for $1\frac{1}{2}$ -in. brass water gauge valve (5). The upper tee was connected by a bushing to $1\frac{1}{2}$ -in. brass tubing leading to the nitrogen cylinder. The lower tee led by the brass tubing to the glass funnel (1) as indicated, and also to an outlet valve (3). All the joints in this part of the apparatus were soldered.

The water gauge tube was of heavy-walled $1\frac{1}{2}$ -in. glass tubing selected for its uniformity of bore. It was fastened into the valves by rubber washers and a litharge and glycerine packing. By means of the water-gauge valves (5) the liquid in the glass tube could be separated from that in the reservoir, and the flow of liquid through the wood disk during a run was measured by its fall in level in the tube. The tube was calibrated in terms of volume to length.

The pressure on the liquid was provided by a cylinder of nitrogen gas connected through a reducing valve to the apparatus. The pressure was registered on a mercury manometer (23) of simple construction. This was planned so that pressures up to seven atmospheres could be read with an

cut to the size of the ring by a circular cutting knife, was forced into the ring from below until the cutting edge of the flange had made a tight seal, so that no liquid could flow around the wood inside the ring. This was found to be very satisfactory, as it was possible to cook wood almost to a pulp before the outer edge of the wood came away from the ring. As the clamp had to be dismantled after each run, it was necessary to have some rapid means of disconnecting it from the rest of the apparatus. This was provided by "cutting" a compression coupling into each of the four tubes leading from the clamp. By unscrewing the four couplings, the clamp could be detached, and it could be re-attached just as quickly by screwing the couplings up again.

(b) The Front-pressure System

The liquid used for penetration was introduced into the apparatus through a glass funnel (1), flowing by gravity through $\frac{1}{8}$ -in. brass pipe past a valve (2) into the cylindrical brass reservoir (4) and the water gauge

error of not more than 1%. The connection of the manometer to the copper tubing of the apparatus was made originally by a litharge and glycerine packing, but this was found to leak, and a more satisfactory union was made by roughening the surface of the copper tubing with a file, then slipping it into the glass tube of the manometer, and packing the joint with medium DeKhotinsky cement. If the cement were heated and moulded carefully, it made a very durable seal.

The liquid from the gauge tube flowed through valve (7) into the clamp, to the front, or high pressure side of the disk, the air in the connections being let out by valve (25). Valve (10) was an outlet for sampling the liquor in the cylinder. Whenever it was desired to blow out or wash out the liquid from the front side of the wood, this was accomplished by opening valves (11) and (25), then forcing through either nitrogen or water by using valves (12) or (13) respectively.

(c) The Back-pressure System

The connections to the back, or low, pressure side of the wood were very similar. One was a very short outlet tube which passed through a cold water bath to an outlet valve (26). The inlet tube connected the back side of the disk with the supply of penetrating liquor, by valve (9), or with a supply of nitrogen or water, by valves (17) and (18), and it also connected by valve (15) with the back pressure manometer (22) and volume equalizer (21). The manometer was similar to that previously described. The volume equalizer was an Alemite grease gun filled with water. Its purpose may be explained very simply. The entire system on the low pressure side of the wood was kept filled with liquid during a run. In order to change the manometer reading, more or less liquid was required in the system; this could be effected by changing the position of the plunger in the grease gun. A small valve connected to the bottom of the gun was found to be necessary to prevent slow leakage of liquid past the plunger.

The flow indicator (20) was of 6 mm. pyrex tubing, with bulbs blown on near the top of each arm. These were to prevent the mercury which was in the bottom of the tube from being blown out of the tube by a sudden flow of liquid. When the back-pressure system was filled and the pressure adjusted, the level of mercury in each arm was the same. If additional liquid were forced into this system through the wood, it caused the mercury levels to change. When the relief valve (26) was opened slightly, the extra liquid escaped and the mercury in the two tubes came to the same level again. In this way, the volume of liquid and the pressure back of the wood could be maintained at a constant value during a run, the penetrating liquid being removed as it came through. This flow indicator was connected to the copper tubing by DeKhotinsky seals as described above.

(d) The Constant Temperature Bath

The clamp was maintained at a constant temperature during a run by a thermostatically controlled bath. This bath was a 14-qt. pail of glycoline,

heated by two 800 watt 110 volt "Calrod" elements, bent to fit the bath. The wiring was arranged to permit power of 100, 200, 400, 800 or 1600 watts being used for heating. Regulation of a part of the current was provided by a DeKhotinsky thermoregulator, connected to an electrolytic condenser. The regulator actuated a small relay which controlled the heating current. Stirring was provided by a $\frac{1}{4}$ h.p. motor driving a propellor blade type stirrer.

Preparation of Materials

The wood used in the experiments recorded in the following pages was white spruce, obtained through the courtesy of the Forest Products Laboratories of Canada. They provided wood of the following types: (a) heartwood of dense structure, thoroughly air dried; (b) heartwood of medium structure, thoroughly air dried; (c) heartwood of porous structure, thoroughly air dried; (d) heartwood of medium structure, freshly cut; (e) sapwood, thoroughly air dried; (f) sapwood, freshly cut. The freshly cut wood was covered with a layer of hot paraffin wax at both ends as soon as it was received, and after any wood was removed for experiments, the remainder was protected in the same way.

In preparing for a forced penetration run, a disk of wood about $1\frac{1}{2}$ in. in diameter and $\frac{7}{8}$ in. thick was sandpapered to the exact thickness desired, 2 cm. This left the surface smooth. The wood was then placed in a small vacuum bottle and the air sucked out for about ten minutes, then distilled water was allowed to rush in surrounding the wood, and the wood was allowed to soak for about a day. Sutherland and Maass (12) found that this preliminary soaking hastened the attainment of equilibrium in the rate of penetration. This was probably due to hydration of the wood. It also allowed the wood to swell freely, so that there would not be the distortion which might occur if the wood swelled in the clamp.

The sulphite liquor was prepared from a good technical grade of lime (testing 99% CaO) and sulphur dioxide supplied by the Matheson Co. This gas was washed with a solution of barium chloride in hydrochloric acid, then with distilled water before it was forced into the lime suspension. In accordance with the usual practice in pulp research, the concentration of lime in the solutions discussed in this paper has been expressed as "combined sulphur dioxide", this being the stoichiometric amount necessary to form calcium sulphite with the lime used. The methods of testing were selected after a brief preliminary survey. The original Winkler method, using iodine and sodium hydroxide, was found to be most satisfactory, and was used throughout the research. The objections to it which Klason (6) raised are not valid for the early stages of a cook. The Sander (9) method, using mercuric chloride solution, was found to give the same results as the Winkler method. The difficulty of getting a well-defined end-point using brom-phenol blue at night was the chief objection to it. The iodate method recommended by Birchard (1) was found to be unsatisfactory.

Procedure

The soaked wood was cut to fit the ring of the clamp, and forced into it until it was tight. The clamp was then assembled, and the bolts tightened firmly, after which the whole clamp was attached to the rest of the penetration apparatus by the four compression couplings. A litre of the solution to be used for penetration was poured into the reservoir of the apparatus, and valve (2) closed. With valve (24) closed, and valves (7) and (25) open, a small pressure of nitrogen was applied to the system, until solution came out of the outlet at valve (25), indicating that the system at the front side of the disk was filled with liquid. Valve (25) was then closed. By opening valve (9) the air at the back of the disk was flushed out at (26), after which valve (9) was again closed.

The clamp and connections on both sides of the wood were then full of liquid, and the apparatus was ready for use in measuring the rate of penetration under differential pressure. It was found by Sutherland (12), however, that after water started to flow through wood, there was a considerable time interval before the velocity of flow came to an equilibrium value. In practically all the runs reported in this paper, this delay was overcome by starting the run at night, leaving penetration to proceed slowly until morning.

After the wood was treated overnight in this way, the actual run was started. The front pressure was adjusted by regulating the reducing valve on the nitrogen cylinder, the back pressure was built up to the desired point by forcing the Alemite gun plunger along its cylinder, and the temperature set by adjusting the thermoregulator in the glycoline bath. The lower water-gauge valve (5) was then shut so that there would be no liquid communication between the reservoir and the gauge tube, but the nitrogen pressure would apply equally to both. Under the differential pressure, the solution was forced through the wood, and by "cracking" valve (26) from time to time, the back pressure could be maintained at the desired value. At definite time intervals, usually of 10 or 15 min., the level of liquid in the gauge tube was noted, from which could be calculated the rate of penetration. After it was evident that the rate was constant, the pressure or the temperature would be changed, and readings again taken until a constant value was obtained.

In some of the runs, analysis was made of the solution which had passed through the wood by collecting a small sample at the outlet tube of valve (26), and titrating a portion of it. In taking such a sample, care was required to ensure that the liquid was thoroughly cooled by passing through a water bath before it came out; otherwise a considerable part of the free sulphur dioxide escaped.

After the run was complete, the liquor was allowed to drain or was blown out of the apparatus, and water forced through to wash out all the tubing of the apparatus. If the wood was required for chemical analysis, distilled water was forced through it until the sulphite liquor was washed out. The clamp was then dismantled.

Experimental Results

The Cooking Action of the Solution

In some preliminary experiments, it was observed that the rate of penetration increased continuously during runs at a high constant temperature, indicating a progressive opening up of the wood, or cooking action. Table I presents results of three runs made on heartwood, of medium penetrability, to find the minimum temperature at which this cooking action could be observed.

TABLE I

RESULTS OF THREE RUNS WITH HEARTWOOD OF MEDIUM PENETRABILITY

Run No. 6			Run No. 7			Run No. 8		
Time, min.	Rate, cc./min.	Temp., ° C.	Time, min.	Rate, cc./min.	Temp., ° C.	Time, min.	Rate, cc./min.	Temp., ° C.
15	0.096	65	15	0.060	90	15	0.078	90
30	0.096	65	31	0.066	90	30	0.090	90
45	0.098	65	81	0.067	90	75	0.095	90
60	0.135	85	120	0.054	90	90	0.102	90
75	0.150	85	176	0.090	130	120	0.103	90
90	0.156	85	191	0.120	130	170	0.156	130
100	0.152	85	241	0.230	130	190	0.230	130
245	0.147	85	251	0.254	130	230	0.404	130
275	0.147	85	303	0.404	130	240	0.434	130
			313	0.434	130	315	0.720	130
			383	0.518	130	335	0.810	130
			393	0.524	130	400	1.080	130

NOTE:—

Run No. 6. Conc. of sol'n:—SO₂: total, 3.49; free, 1.90; combined, 1.59%. Press.:—front, 7 atm.; back, 5 atm.

Run No. 7. Conc. of sol'n:—SO₂: total, 2.90; free, 1.80; combined, 1.10%. Press.:—front, 7 atm.; back, 6.5 atm.

Run No. 8. Conc. of sol'n:—SO₂: total, 3.04; free, 1.99; combined, 1.05%. Press.:—front, 7 atm.; back, 6 atm.

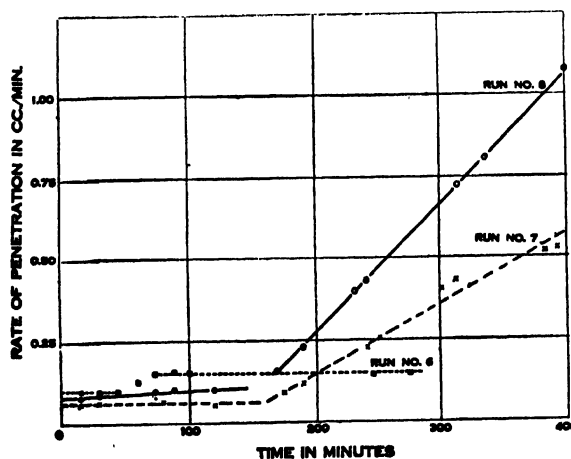


FIG. 3. Minimum temperature of cooking action as indicated by rate of penetration.

The data from these runs are plotted in Fig. 3. In run No. 6, there was no indication of appreciable cooking action at temperatures up to 85° C., but at 90° C., especially in run No. 8, there seemed to be a slow gradual increase. At 130° C., there was a rapid opening up of the wood. It is interesting to note that in run No. 8, where the pressure differential was twice that in No. 7, the rate of penetration is also practically double.

The Effect of Sulphur Dioxide Concentration

Four disks of wood from the same cylinder were tested for the effect of sulphur dioxide concentration by measuring the rate of penetration under similar conditions except for the variation in concentration of the liquor. In three of the runs the concentration of combined sulphur dioxide was constant at 1.5%, that of total sulphur dioxide being 6.50, 5.06 and 3.55%. Distilled water was used for the fourth disk. The results of the four runs are summarized in Table II, and are plotted in Fig. 4.

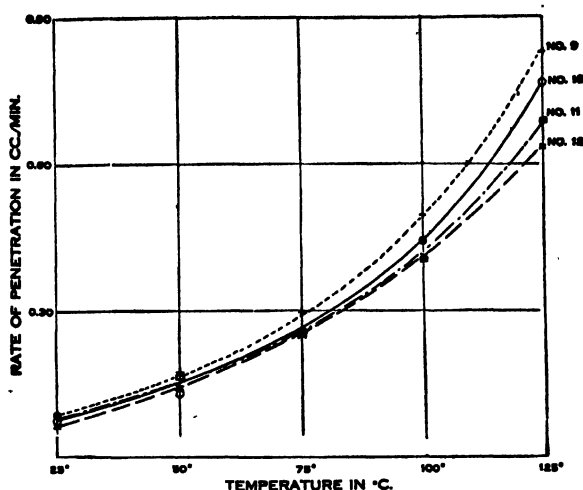


FIG. 4. Effect of sulphur dioxide concentration. No. 9, 6.50% SO₂; No. 10, 5.06% SO₂; No. 11, 3.55% SO₂; No. 12, 0.0% SO₂.

TABLE II
EFFECT OF SULPHUR DIOXIDE CONCENTRATION

Temp., ° C.	6.50% SO ₂	5.06% SO ₂	3.55% SO ₂	Water
	Rate of penetration, cc./min.			
25	0.093	0.082	0.092	0.070
50	0.181	0.132*	0.153	0.157
75	0.297	0.268	0.265	0.262
100	0.495	0.442	0.405	0.415
125	0.830	0.770	0.690	0.635

*Doubtful.

The rate of penetration was greater for a larger percentage of sulphur dioxide, the difference being more noticeable at higher temperatures. Where water was used as the penetrating fluid, there was little difference from the run using 3.5% sulphur dioxide except at 125° C. This increased differential at 125° C. should doubtless be explained as a cooking action.

The Effect of the Formation of a Precipitate

The influence of the precipitation of a lime salt on the rate of penetration of sulphite liquor was also studied. Considerable attention was given to this part of the subject as its industrial importance can hardly be over-emphasized. It is well known that when some solutions of calcium bisulphite are heated, a precipitate of a lime salt forms, and the following experiments

were done to find out what plugging effect this precipitate might have. A preliminary run (No. 13) was made using a high percentage of lime in the liquor. The results are shown in Table III.

TABLE III
EFFECT OF A HIGH PERCENTAGE OF LIME IN THE LIQUOR (RUN No. 13)

Temp., ° C.	75	85	95	105	115	115
Rate, cc./min.	0.150	0.183	0.223	0.286	0.313 (at start)	0.210 (after 1 hr.)

NOTE:—Wood: air-dry heartwood, medium penetrability. Conc. of sol'n:—SO₂: total, 3.12; free, 1.71; combined, 1.41%. Pressure:—front, 7 atm.; back, 5 atm.

At a temperature of 115° C., there was evidently a plugging effect due to the formation of a precipitate. When the clamp was opened at the end of the run, there was a dense layer of precipitate all over the surface of the wood. The precipitate was a mat of small crystals, which liberated sulphur dioxide with acid. A test sample of the liquor had shown a precipitate at approximately 90° C., but there was no evidence in the penetration run of the formation of a precipitate until above 105° C. During the other runs, this same phenomenon occurred frequently—no evidence of a precipitate under conditions which should cause its formation. Once the precipitate had formed, a heavy deposit of it occurred. This is in agreement with the results of Gurd (3), who has found that in an SO₂-H₂O-CaO system, the solution may exhibit metastability over a temperature range as great as 25° C. before a precipitate of a calcium salt forms.

When it was established that the precipitate exercised a plugging action, two lines of investigation were followed. In one, the effect of the porosity of the wood was studied, and in the other, the location in the wood of the plugging agent. Samples of wood of three degrees of porosity were selected and given the usual preliminary treatment. The rate of penetration at a temperature slightly below the precipitation point of the solution was then measured over a period of one to two hours, at the end of which time the temperature was raised quickly to a point above the precipitation temperature and readings were taken again for several hours. In these runs (Nos. 14, 15 and 18) the solution contained 3.60% total SO₂, of which 1.98% was free SO₂, the approximate precipitation point being 80° C. A summary of the runs is given in Table IV.

In each of these runs a layer of calcium sulphite was found over the surface of the wood at the end of the run, proving that the precipitate had formed. Where the wood was very dense, the precipitate apparently had little effect on the rate of penetration, as the change observed was about that expected from the change in temperature. The wood of medium porosity was apparently slightly plugged, and the continuous decrease in rate at the higher temperature suggested an increasing plugging action. With the very porous wood, the decreased rate of penetration at the higher temperature could be ascribed only to a pronounced plugging effect. It is suggested that the open-

TABLE IV
EFFECT OF THE POROSITY OF THE WOOD

Run No. 14			Run No. 15			Run No. 18		
Time, min.	Temp., °C.	Rate, cc./min.	Time, min.	Temp., °C.	Rate, cc./min.	Time, min.	Temp., °C.	Rate, cc./min.
30	65	0.018	30	70	0.049	30	65	0.050
60	65	0.022	60	70	0.055	60	65	0.060
90	65	0.021	90	70	0.057	90	65	0.063
120	65	0.023	120	70	0.052	120	65	0.060
0	100	—	0	110	—	0	100	—
30	100	0.041	30	110	0.080	30	100	0.054
60	100	0.039	60	110	0.082	60	100	0.049
90	100	0.032	90	110	0.078	130	100	0.046
120	100	0.043	120	110	0.076	150	100	0.049
150	100	0.042	150	110	0.066	180	100	0.051
180	100	0.040	180	110	0.067			
240	100	0.043						

NOTE:—

Run No. 14. Wood: air-dry heartwood, low penetrability. Pressure:—front, 7 atm.; back, 5 atm.

Run No. 15. Wood: air-dry heartwood, medium penetrability. Pressure:—front, 7 atm.; back, 5 atm.

Run No. 18. Wood: air-dry heartwood, very porous. Pressure:—front, 7 atm.; back, 6.75 atm.

ings of the bordered pits in the dense wood were so small that they remained the limiting factor in the rate of flow, even with the precipitate present, but with the more porous wood, the openings of the bordered pits were sufficiently large that the particles of precipitate did effect the rate of flow.

If the plugging action is more pronounced with more porous wood, there should be a much more noticeable effect with sapwood, and two runs (Nos. 20 and 21) were made to check this hypothesis. The open structure of sapwood makes it impossible to use a large pressure differential. In these runs, the differential was the hydrostatic head of the solution between the level in the gauge tube (Fig. 1, 6) and the outlet valve (Fig. 1, 26). As the liquid flowed through the wood, this head decreased and the rate showed a corresponding diminution. As a measure of the porosity of the wood, the ratio of rate of flow/hydrostatic head was used.

In the first run, the porosity of the wood was determined at room temperature (18° C.), then the wood and solution were heated to 70° C. for 30 min., and the porosity again determined as soon as the wood could be returned to room temperature. This showed the effect of cooking without the solution being allowed to precipitate. The second run differed from the first in that the wood and solution were heated to 100° C. for ten minutes between the two periods when the porosity was being determined. This temperature was sufficiently high to cause the precipitation of the lime salt.

In run No. 20 the heating to 70° C. for 30 min. was selected in an attempt to cause about the same cooking action as the heating to 100° C. for 10 min. in run No. 21. In this way, some allowance could be made for the cooking

TABLE V
RESULTS OBTAINED WITH SAPWOOD

Time, min.	Vol. in cc./min. Hydrostatic head	
	Run No. 20	Run No. 21
5	0.0430	0.0646
10	0.0409	0.0576
15	0.0398	0.0576
20	0.0389	
25	0.0389	
30	0.0404	
	Heated to 70° C. for 30 min., then cooled to room temp.	Heated to 100° C. for 10 min., then cooled to room temp.
5	0.0794	0.0247
10	0.0748	0.0223
15	0.0732	0.0218

effect necessarily occurring at the precipitation temperature. From the results obtained in former runs, it was likely that the cooking effect at 100° C. would be greater than at the lower temperature, even for the longer time. In run No. 20, due to the effect of cooking, the ratio of rate/hydrostatic pressure was very nearly double, but in run No. 21, with as great cooking effect, the ratio dropped to little more than one-third its former value. If we assume that the cooking would open both blocks of wood to the same extent, the

ratio should have doubled in run No. 21, whereas it actually dropped to one-third. This decrease confirmed the evidence of the other runs that in wood of open texture, where a large pressure differential was not required, the precipitation of a calcium salt did cause a decrease in the rate of flow of liquid.

In many of the runs where plugging was observed, it was noted at the end of the run that a layer of calcium sulphite was covering the wood surface. The next three runs were made to find out whether the plugging action was at the surface, or through the whole of the wood. In order that there would not be any effect due to particles of precipitate settling on the upper surface of the wood due to gravity, the clamp was reversed so that the inflow of sulphite liquor was from below. Any precipitate which might form in the liquor before it reached the wood would thus settle away from the wood. To prevent precipitation in the feed tube where it passed through the hot oil, the feed tube was covered with a small lead pipe through which cold water was kept running. In this way, the sulphite liquor was brought to the clamp at full strength, without any loss by precipitation.

In these runs, the rate was measured at definite temperatures before precipitation occurred, then the temperature was raised above the precipitation point and the decrease in rate noted. The clamp was then dismantled. Both upper and lower surfaces of the wood were scraped free of deposit, and a little dilute hydrochloric acid passed quickly over the surface and washed off, the acid being in contact with the wood about 15 sec. In this way it was hoped to remove all surface plugging but leave any sulphite precipitate in the wood. The clamp was then reassembled, and the rate of penetration determined at the same temperature as before.

TABLE VI

RESULTS OF EXPERIMENTS WITH CLAMP REVERSED; INFLOW OF SULPHITE LIQUOR FROM BELOW

Run No. 22		Run No. 23			Run No. 24		
Temp., ° C.	Rate, cc./min.	Time, min.	Temp., ° C.	Rate, cc./min.	Time, min.	Temp., ° C.	Rate, cc./min.
30	0.025	20	60	0.078	20	85	0.975
50	0.042	40	60	0.069	35	85	0.877
70	0.077	60	60	0.069	51	85	0.870
90	0.125	0	100	—	0	110	—
110	0.106	10	100	0.186	5	110	0.810
		20	100	0.180	10	110	0.510
		65	100	0.164	25	110	0.210
		75	100	0.164	40	110	0.120
		175	100	0.110	55	110	0.090
		275	100	0.104			

The clamp was then dismantled, the surfaces of the wood cleaned, the clamp reassembled and filled, and the penetration continued.

30	0.075	10	60	0.290	10	85	1.530
50	0.117	20	60	0.262	20	85	1.058
		35	60	0.235	30	85	0.690
		55	60	0.174	40	85	0.450
		120	60	0.102			
		420	60	0.075			

NOTE:—

Run No. 22. Wood:—heartwood, medium penetrability. Approx. precipitation point of solution in test, 90° C.

Run No. 23. Wood:—heartwood, medium penetrability. Approx. precipitation point of solution in test, 70° C.

Run No. 24. Wood:—heartwood, very porous. Approx. precipitation point of solution, 85° C.

In all three runs, after the precipitate had been removed, the rate of flow was much greater than it had been at the same temperature before precipitation occurred (Table VI). This could be explained only as a cooking action. The decreased rate of flow observed due to the plugging action of the precipitate does not remain when the surfaces are cleaned, establishing definitely that the most of the plugging action is at the surface layers.

In runs Nos. 23 and 24, the gradual decrease in rate after the wood had been scraped was due to the formation of a new layer of precipitate which formed on the wood. Evidently the temperature at which the precipitate formed in the test sample was higher than the true precipitation temperature, and after a few crystals were present to act as a start, the precipitate formed at a lower temperature. This was an example of the tendency to supersaturation, previously mentioned.

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A LABORATORY COMPARISON OF THE DETERGENT EFFICIENCIES OF LAUNDRY SOAP BUILDERS¹

By O. M. MORGAN²

Abstract

The detergent efficiencies of four common laundry soap builders have been measured and compared on a laboratory scale. Their order of decreasing efficiency when both concentration and actual washing efficiency are considered is caustic soda, soda ash, sodium metasilicate and trisodium phosphate. When actual washing efficiency alone is considered the order is sodium metasilicate, caustic soda, trisodium phosphate, and soda ash. In all experiments the soap concentration was 0.1%.

pH values of the built soaps are given over a wide range of concentration. All soap builders do not exert their optimum detergent effect at the same pH value.

Two of the alkalis, namely caustic soda and sodium metasilicate, increased the lathering power of the soap notably.

Although a knowledge of the chemical and physical characters of alkalis and of their effects on the physical properties of soap solutions is indicative of their detergent effect, yet actual washing tests constitute the only satisfactory final criterion of the detergent efficiency of soap builders. In previous papers (2, 3) there was described a technique for determining in quantitative terms, by means of a small-scale laboratory washing operation, the detergent efficiency of washing procedures. The present paper deals with the application of this technique to a comparison of a number of common alkalis used as soap builders. In a following paper will be given the results of a comparison of the same builders in a full-scale trial in a power laundry.

Previous Work

Alkalis used as soap builders in the power laundry industry offer a field for comparative work. To date this field has been exploited only on laboratory scale. There are very few, if any, cases on record where the exploitation has reached full-scale laundry plant proportions. This is unfortunate since laboratory and plant results do not always coincide. Rhodes and Bascom (4) found that by using a 0.25% soap at 40° C. with added alkali the detergent action first increased and then decreased with increasing alkalinity. With all builders studied the maximum detergent effect was found at a pH of 10.7.

Baker (1) has made one of the best contributions in recent years by a series of fairly simple experiments in which he considers pH, wetting ability, lathering power, and the stability of emulsification produced by soap builders. Snell (5) in a paper dealing with the manufacture and use of trisodium phosphate, gives some comparative figures of pH and alkalinity for several soap builders.

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In later papers (6, 7) Snell discusses the initial available alkalinity of all the common alkalis used as soap builders. In addition to this their effect on the lowering of interfacial tension between water and oils has been determined.

In a previous publication by the writer (2) a quantitative method for measuring the detergent action of laundry supplies was discussed. Method rather than application to full-scale practice was stressed and only neutral soaps were studied.

Experimental

Before attempting actual full-scale laundry plant experiments to determine and compare the detergent efficiency of the common builders, the method was tried out in this laboratory on small scale.

The procedure in this set of experiments was to wash standardly soiled cloth, similar to that previously tested (2, 3), with neutral soap to which had been added increasing amounts of the commonly used soap builders. The increase in reflecting power, or brightness, of the soiled fabric was considered as the criterion of the washing efficiency. This was measured with a Zeiss Pulfrich photometer. The washing was effected in the experimental wash wheel and the same technique was used as previously discussed (2, 3); the temperature of the wash was 50° C. (122° F.), and the soap concentration was 0.1%, and the time of one wash was 10 min. Five consecutive washes were run with each concentration of builder. The reflecting power of the soiled fabric was measured after each wash.

Experimental Results

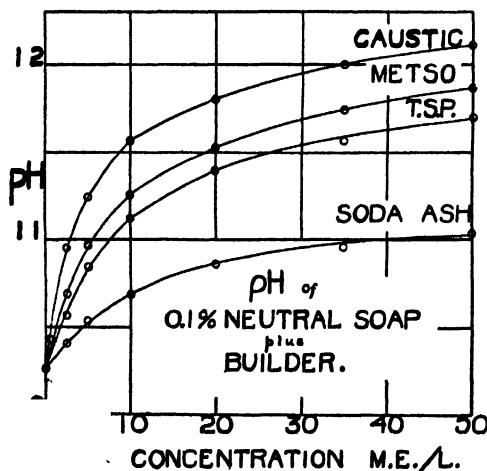


FIG. 1. pH values of 0.1% neutral soap plus builder.

Electrometric pH measurements on the built soaps used in these experiments were made prior to the washing tests. One experimenter (4) has claimed a direct relation between pH and detergency and it was thought desirable to check these results. This phase of the work will be discussed in a later section. The pH data are given in Table I and illustrated graphically in Fig. 1. For purposes of comparison the concentrations of each builder are given in milli-equivalents per litre. For more practical purposes they are also given in weight per cent.

TABLE I
pH VALUES OF 0.1% BUILT SOAP SOLUTIONS

Concentration of alkali M.E./l*	Caustic soda		Soda ash		Trisodium phosphate		Sodium metasilicate		Borax	
	Wt. %	pH	Wt. %	pH	Wt. %	pH	Wt. %	pH	Wt. %	pH
0.5	.002	10.43	.002	10.26	.006	10.28	.005	10.30	.005	10.11
2.5	.010	10.96	.013	10.41	.030	10.57	.026	10.69	.025	9.64
5.0	.020	11.25	.026	10.54	.060	10.85	.052	10.97	.050	9.47
10.0	.040	11.57	.052	10.69	.120	11.12	.104	11.26	.100	9.38
20.0	.080	11.80	.104	10.86	.240	11.40	.208	11.53	.200	9.34
35.0	.140	12.00	.182	10.94	.420	11.56	.364	11.74	.350	9.33
50.0	.200	12.12	.260	11.03	.600	11.69	.520	11.87	.500	9.33

*pH of neutral soap used = 10.24.

NOTE:—For purposes of clarity the concentration term milli-equivalent per litre is explained here. Considering soda ash as an example: chemical formula, Na_2CO_3 ; molecular weight, 106 gm. (approx.); equivalent weight, 53 gm., 1 milli-equivalent = $\frac{53}{1000} = 0.053$ gm. Hence a concentration of one milli-equivalent per litre is 0.053 gm. of soda ash dissolved in water and diluted to 1000 cc.

From these results it will be noted that for a given concentration of builder the alkalinity falls in the following order: caustic soda, sodium metasilicate, trisodium phosphate, sodium carbonate, and borax.

Table II contains the data derived from the washing experiments. The

TABLE II
DETERGENT EFFICIENCY DATA

Alkali	M.E./l	Wt. per cent	pH	S	E
Sodium carbonate	0.0	0	10.24	31.1	0.0
	1.25	0.0065	10.34	38.8	24.8
	5.0	0.026	10.55	40.0	28.6
	12.5	0.065	10.75	38.5	23.8
	25.0	0.130	10.89	35.8	15.1
Trisodium phosphate	0.0	0.0	10.24	29.8	0.0
	2.5	0.030	10.57	33.1	11.1
	5.10	0.060	10.85	38.7	29.8
	10.0	0.120	11.12	38.7	29.8
	30.0	0.360	11.52	32.4	8.7
Sodium metasilicate	0.0	0.0	10.24	27.6	0.0
	2.5	0.026	10.69	35.2	21.8
	5.0	0.052	10.97	38.2	38.4
	15.0	0.156	11.42	36.8	33.3
	40.0	0.416	11.80	32.0	19.6
Caustic soda	0.0	0.0	10.24	30.0	0.0
	0.5	0.002	10.43	34.6	15.3
	1.0	0.004	10.63	36.9	23.0
	2.0	0.008	10.87	39.4	31.3
	5.0	0.020	11.25	38.8	29.3
	10.0	0.040	11.57	34.4	14.7
Borax	0.0	0.0	10.24	30.5	0.0
	0.5	0.005	10.11	31.4	3.6
	2.5	0.025	9.64	30.6	—
	10.0	0.10	9.47	31.4	3.6
	20.0	0.20	9.34	35.2	12.1

first column shows the builder used. The second and third columns give the concentrations of builder used in milli-equivalents per litre and in weight

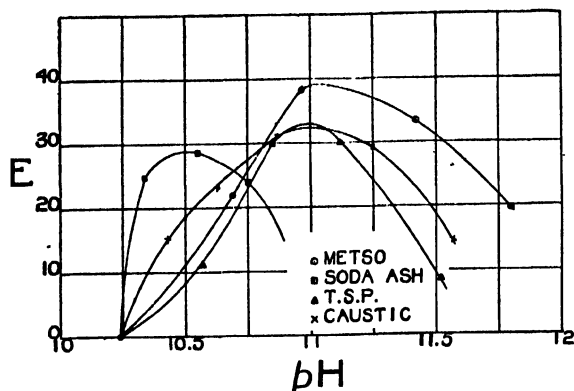


FIG. 2. Relation between the pH of the washing solution and its washing efficiency.

per cent respectively. The fourth column gives the pH value of the wash liquor with which the washing was performed. The fifth column gives the values of S which is explained below. The S value for zero concentration of builder is the B value which is also explained below. The B values vary slightly depending on the batch of soiled cloth used. The sixth column gives the detergent efficiency value

E which is really the increase in efficiency produced by the presence of the alkaline builder in the soap solution. This is calculated from the formula

$$E = 100 (B - S) / S$$

as originally used by Rhodes and Bascom (4) where, B = the increase in brightness of the soiled cloth after five washes with 0.1% soap solution to which builder has been added; S = the increase in brightness of the soiled cloth after five washes with 0.1% neutral soap. This mode of calculation gives neutral soap an E value of zero.

These results are shown graphically in Figs. 2 and 3 where E is plotted against concentration pH and respectively. The data for borax have been omitted from these graphs. Borax does not increase the detergent efficiency of a soap but may possibly have some use as a buffering agent or a water softener. However these properties will not be dealt with here.

Possibly the simplest method of surveying the above data is by means of Table III.

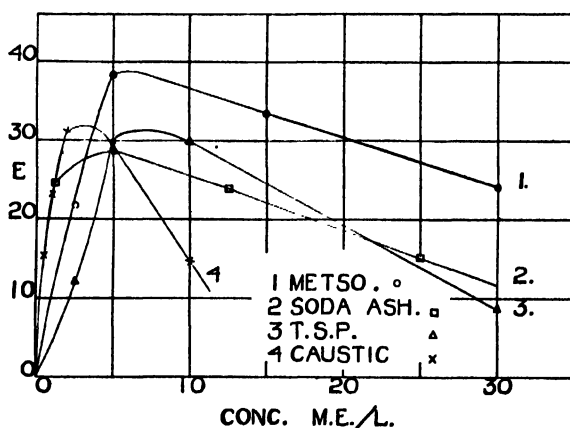


FIG. 3. Relation between concentration of builder and washing efficiency.

TABLE III

SURVEY OF DETERGENT DATA

Alkali	Conc. for maximum efficiency %	E	pH	Efficiency index	Pound efficiency index
Soda ash	0.026	28.6	10.5	1100	2.9
Trisodium phosphate	0.060	31.4	10.9	523	6.1
Sodium metasilicate	0.052	39.0	11.0	750	4.2
Caustic soda	0.010	32.0	10.9	3200	1

The efficiency index given in column 5 of Table III is obtained by dividing the efficiency E by the concentration. This allows a comparison of the efficiencies per unit of concentration. A high index value indicates a high efficiency. In column 6 this information is expressed in another way. The amount of other builders that will do the same work as one pound of caustic soda is calculated.

Discussion of Results

In discussing these results the laundryman is naturally interested in what the comparative costs will be with different builders and the quality of the work that will be turned out. The chemist wishes to determine why certain results were obtained. In the former case it is impossible to give very definite answers until the work has progressed further and reached plant scale. The results are quite indicative however that differences in builder efficiencies do exist. When optimum concentration is linked up with actual washing efficiency these differences become quite pronounced. In rank of decreasing efficiency the builders fall in the order of caustic soda, sodium carbonate, sodium metasilicate and trisodium phosphate. The latter two alkalies are not widely different.

When washing efficiency alone is considered the order of decreasing merit is sodium metasilicate, caustic soda, trisodium phosphate, and soda ash. The factor effecting the change in order of merit is the concentration. Rhodes and Bascom (4) give the order of decreasing efficiency as soda ash, trisodium phosphate and caustic soda. This is a complete reversal of the present findings. However the data of Rhodes and Bascom (4) may be questioned for the following reason. Their results show caustic soda to have little or no effect in increasing the detergent efficiency. In full-scale plant practice this is not the case since the writer knows of a number of Canadian laundries using caustic soda as a soap builder with excellent results. Test bundles issued from this laboratory and run in these plants have turned out extremely well. Any experienced laundryman knows that caustic soda is a good soap builder but requires very accurate control. This point is proved in Fig. 3 where caustic soda is shown to have a very sharp efficiency peak.

It will be noted that trisodium phosphate, sodium metasilicate, and caustic soda exhibit their maximum detergent efficiencies at approximately the same pH values, namely 10.9–11.0; at variance with this soda ash exhibits its maximum efficiency at a pH value of 10.5. This is a point not readily explainable. However it would tend to indicate that all builders are not under optimum detergent conditions at the same pH value. Plant experiments should throw some light on this matter.

In considering all the points brought forward in this work several limitations must not be overlooked. One type of soil has been used. Even though this soiling mixture is quite representative of the type of dirt encountered in the laundry it must not be considered as necessarily representative of all conditions. All results obtained have been dependent on the reactions of this soil to the washing process. It has merely provided a standard means of comparison. Nevertheless it is felt that this soil is representative in the majority of cases. Special and involved cases will require particular attention as they appear.

In the present work the optimum builder concentrations have been obtained using a soap concentration of 0.1%. It has been noted that the lathering power of this soap is affected to different degrees by different builders, notably sodium metasilicate and caustic soda. It is quite possible that lower soap concentrations would be found adequate when using these builders. This will be determined in plant tests.

Acknowledgments

The writer wishes to thank Mr. C. W. Davis of these laboratories for analyzing the alkalies and soap used in this work.

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THE DETERMINATION OF MOISTURE IN HONEY BY THE HYDROMETER METHOD¹

By H. D. Chataway²

Abstract

The hydrometer method of testing honeys has been shown to give results as satisfactory as those obtained by other methods provided that; (1) a large sensitive hydrometer is used, (2) the percentage of moisture is calculated from a proper honey table and not from the usual sugar tables, and (3) a shallow layer of water is added to the surface of the honey after the hydrometer has been introduced. A suitable, specially constructed hydrometer is described, and a table of true honey densities has been drawn up. For accurate work the observed densities should be corrected for the slight error which would otherwise arise from the smaller buoyancy thrust of the water layer as compared with a honey layer of the same thickness.

Introduction

In a previous paper (1) methods for the determination of moisture in honey by means of both refractive index and viscosity measurements were described in detail. It was stated there that, although the hydrometer method as commonly used gave very poor results, the possibilities of this method would be explored. This has been done, and the results are given below.

One of the obvious disadvantages of the hydrometer method is that it requires a fairly large sample of honey, depending on the size of the instrument. For this reason in designing a special hydrometer for honey purposes an attempt was made to keep the size as small as possible. However, since a difference of 0.2% moisture corresponds to only 0.09° Bé., it was clear that a hydrometer to give accurate results would need to have a wide open scale, and this set a limit to the possible compactness of the instrument. Thus an instrument (Fig. 1) of minimum dimensions suitable to the purpose proved to be one having an over-all length of 32 cm., a maximum diameter of 1.7 cm., a stem diameter of approximately 5 mm., and a scale 11.7 cm. long graduated between 37 and 44.5° Bé. About 5 cm. might have been saved in the length of the stem by omitting the graduations from 37 to 40° Bé., since this part of the stem was not required for testing the range of honeys reported in this paper, but would be needed for work on thin nectar honeys. The instrument also had a thermometer graduated from 60° to 140° F. built into it. It was made to order, and both the hydrometer and thermometer scales have been standardized and found to be without appreciable error.

It is well known that hydrometer tests on viscous materials such as honey give very inaccurate results. The hydrometer appears to stick at positions quite other than the true equilibrium ones. Thus, in using the above instrument, although there could be no very great error in reading its actual position, repeated tests gave very erratic results no matter what length of time was allowed for the instrument to come to equilibrium.

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Searching for the exact cause of this it was easy to see that if the stem has once been wetted with honey to a point above the equilibrium one the hydrometer in rising to its true position will be weighed down by a layer of honey clinging to the emerging portion of the stem. A layer of water was therefore placed on the top of the honey in order to supply a non-clinging surface, and this simple expedient* proved entirely successful. Near its equilibrium point, the hydrometer moves slowly so that the water dissolves the adhering film of honey, keeping the stem clean. As a result the readings obtained were found to be accurate and dependable.

Experimental

The densities of a number of honeys, obtained for the most part from ordinary commercial sources, were measured using the above hydrometer, and at the same time the moisture contents were determined by the refractive index method using the special honey tables developed previously. In the case of ten honeys, readings were taken not only on the original honeys but also on the same honeys after they had been diluted, and in the case of three honeys readings were similarly taken after the honeys had been concentrated at moderate temperatures. Nothing in the least abnormal could be discovered about these readings on diluted and concentrated honeys, and they have therefore not been kept separate from the others in the table given. Three of the honeys (designated B in the table) were medium flavored buckwheat honeys, but they did not give decidedly abnormal results, probably due to the fact that abnormalities in the relation between density and moisture content would be reflected in the relation between refractive index and moisture content.

In determining the densities, the honeys were heated to 120° F. in a hot-air oven and when completely liquefied were thoroughly mixed and a sample poured into an upright glass cylinder, 35 mm. in internal diameter and approximately 30 cm. in height. The hydrometer was introduced into the sample and then 5 cc. of water was poured on the surface. After 10 or 15 min. the apparent density, as given by the top of the water meniscus, was read, and the temperature noted. These readings were repeated two or three times and then the density readings were corrected to 68° F., whereupon the individual values very rarely differed from one another by more than 0.12° Bé.

In order to determine the temperature correction factor, 15 readings were carefully taken on a sample of honey as the temperature was dropping from 127 to 84° F. On plotting these it was found that 1° F. corresponds to a difference of 0.02° Bé.



FIG. 1. Honey hydrometer drawn to scale. (a) Sealing wax; (b) lead shot; (c) mercury.

*Suggested by the author's colleague, D. F. Stedman.

It was also necessary to correct for the effect of the water layer due to the difference in density of water and honey. Since this correction is not large no appreciable error is introduced by calculating it on the assumption that the specific gravity of all honeys is 1.40. From this it would follow that the buoyancy thrust of the water layer would be $\frac{1}{14}$ less than that of a layer of honey of the same thickness, namely 5 mm. Actually this thickness amounted to a distance on the scale reading of the hydrometer of 0.32° Bé, $\frac{1}{14}$ of which is equal to a difference of 0.09° Bé. The observed readings had therefore to be increased by 0.09° Bé in order to give the true densities of the honeys.

TABLE I

EXPERIMENTAL RESULTS ON COMMERCIAL HONEYS

% Water calcd. from ref. index	Observed Beaumé readings	Corrected Beaumé readings	% Water calcd. from Table II	Diff. between columns 1 and 4
15.61	43.51	43.60	15.72	+ .11
17.36	42.87	42.96	17.26	-.10
17.19	42.98	43.07	17.00	-.19
13.68	44.39	44.48	13.55	-.13
16.40	43.20	43.29	16.48	+ .08
19.13	42.00	42.09	19.07	-.06
16.66	43.17	43.26	16.55	-.11
17.00	42.93	43.02	17.12	+ .12
16.84	43.11	43.20	16.69	-.15
19.06	42.22	42.31	18.63	-.43
15.64	43.60	43.69	15.47	-.17
14.16	44.22	44.31	13.96	-.20
17.27	42.89	42.98	17.21	-.06
17.46	42.70	42.79	17.63	+ .17
18.00	42.51	42.60	18.04	+ .04
17.56	42.77	42.86	17.47	-.09
18.29 (B)	42.25	42.34	18.57	+ .28
18.40 (B)	42.18	42.27	18.71	+ .31
17.84 (B)	42.44	42.53	18.19	+ .35
17.34	42.84	42.93	17.33	-.01
18.15	42.48	42.57	18.10	-.05
16.01	43.28	43.37	16.29	+ .28
17.46	42.71	42.80	17.61	+ .15
19.00	41.92	42.01	19.23	+ .23
19.07	41.93	42.02	19.21	+ .14
17.58	42.80	42.89	17.41	-.17
16.50	43.20	43.29	16.48	-.02
18.33	42.40	42.49	18.27	-.06
18.23	42.32	42.41	18.44	+ .19
18.27	42.44	42.53	18.19	-.08
19.11	42.04	42.13	18.98	-.13
18.78	42.12	42.21	18.84	+ .06
18.53	42.22	42.31	18.63	+ .10
19.58	41.65	41.74	19.72	+ .14
16.42	43.11	43.20	16.69	+ .27
20.22	41.45	41.54	20.10	-.12
15.16	43.71	43.80	15.22	+ .06
14.72	43.75	43.84	15.11	+ .39

TABLE II

RELATION OF MOISTURE CONTENT TO DENSITY*

Water, %	Density at 68° F. on Beaumé scale	Water, %	Density at 68° F. on Beaumé scale	Water, %	Density at 68° F. on Beaumé scale	Water, %	Density at 68° F. on Beaumé scale
13.0	44.70	15.0	43.89	17.0	43.07	19.0	42.12
.2	.62	.2	.81	.2	42.98	.2	.02
.4	.54	.4	.73	.4	.89	.4	41.92
.6	.46	.6	.65	.6	.80	.6	.82
.8	.38	.8	.57	.8	.71	.8	.71
14.0	.30	16.0	.49	18.0	.62	20.0	.60
.2	.21	.2	.41	.2	.52	.2	.49
.4	.13	.4	.32	.4	.42	.4	.38
.6	.05	.6	.23	.6	.32	.6	.27
.8	43.97	.8	.15	.8	.22	.8	.16
						21.0	.05

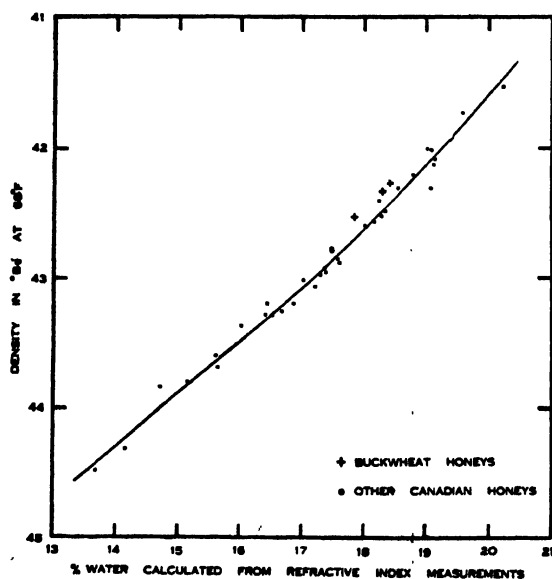
*Correction for temperature = $-0.02^\circ \text{Bé per } ^\circ \text{F.}$ 

FIG. 2. Curve indicating degree of accuracy obtainable by improved hydrometer method of determining moisture in honey.

were obtained. The differences between the values in the first and fourth columns therefore give the magnitude of the experimental error characteristic of this method of determining moisture contents. These differences are given in Column 5.

The results are given in the first three columns of Table I and in Fig. 2. From these, Table II has been drawn up showing the true relation between honey densities and percentages of moisture. As in the case of the refractive indices it will be seen that the densities of honeys are considerably less than those of sugar syrups of the same moisture contents, the differences being such as to cause an error of from $1\frac{1}{2}$ to 2% in the calculated moisture content if the ordinary sugar tables are used. Calculating back from the observed densities and the values given in Table II, the values given in the fourth column of Table I

Note on Viscosity Method

While carrying out the above work, moisture determinations by the viscosity method, described in the previous paper (1), were also made on the honey samples. The results, while they do not in any way discredit the viscosity method of determining moisture, emphasize the fact that in making the arbitrary measurements of viscosity it is essential to use a tube of exactly the internal diameter originally specified, *i.e.*, 15 mm. A difference of 1 mm. leads to an average error of 0.1% moisture.

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THE PREPARATION OF β -CHLOROPROPIOPHENONE¹

By C. F. H. ALLEN,² H. W. J. CRESSMAN³ AND A. C. BELL³

Abstract

A suitable, inexpensive apparatus and procedure for the preparation of β -chloropropiophenone from ethylene and benzoyl chloride in the presence of aluminium chloride are outlined. Several homologues are also described.

The use of vinyl phenyl ketone in a certain type of addition reactions has been the subject of several recent papers (1, 2, 3). The most satisfactory source of the unsaturated ketone was found to be β -chloropropiophenone (1, 5, 7, 9, p. 845), but the best known method for preparing the latter involved so many steps it seemed advisable to try to devise a more suitable procedure.

Norris and Couch (18) described the preparation in small amounts, of vinyl phenyl ketone by the Friedel-Crafts reaction, from benzoyl chloride and ethylene in the presence of anhydrous aluminium chloride. From Kohler's earlier study of vinyl phenyl ketone (12) it was evident that β -chloropropiophenone should be an intermediate product, from which the unsaturated ketone was produced by loss of hydrogen chloride during the distillation; this was later found to be the case (17). Accordingly the problem resolved itself into devising a suitable apparatus for carrying out the reaction, which may be represented by the outline



However, it is not as simple as it appears. The benzoyl chloride and aluminium chloride form a double compound, insoluble in most solvents except ethyl bromide (17). The ethylene must be introduced in some way so that it becomes well-mixed with the solution, not used in too great an excess for economic reasons, and be available for the duration of the run. The β -chloroketone is easily decomposed by heat, so its isolation from the reaction mixture after decomposition of the aluminium chloride complex must be carefully accomplished.

At first sight it seemed that the easiest way to speed up the very slow reaction was to operate under pressure. When this was done, very little of the desired product resulted, but much thick, brown oil. In view of the work of recent investigators (15, 21) on the production of synthetic lubricating oil, the latter was probably polymerized ethylene. It was finally found that only enough pressure to get the gas into the reaction vessel was needed. The details of the construction of the apparatus that finally proved satisfactory and its operation are given in the experimental part.

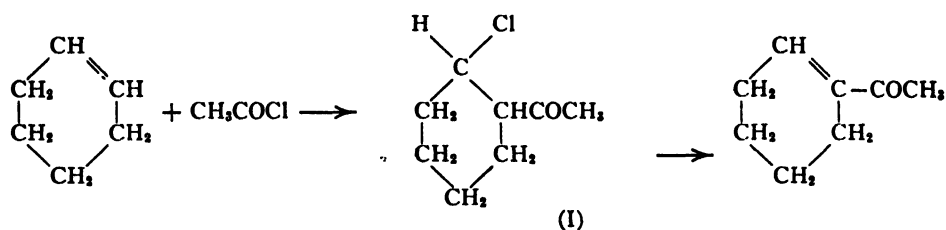
¹ Manuscript received March 14, 1933.

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The Friedel-Crafts reaction has been applied to non-benzenoid hydrocarbons to a relatively slight extent until recently. Krapivin (14) prepared aliphatic unsaturated ketones from acetyl chloride or bromide and propylene, cyclopropane, isobutylene, hexylene, heptylene, and octylene. Darzens (8) recorded that acetyl chloride did not react with cyclohexane in the presence of anhydrous aluminium chloride, but with cyclohexene a saturated chloro-ketone (I) resulted; stannic chloride gave a better yield.



Since hydrogen chloride could readily be eliminated, the reaction would simulate that of an aromatic compound and suggest that the production of ketones from aromatic hydrocarbons by the Friedel-Crafts reaction proceeds through an intermediate addition product; this mechanism has been advocated by Wieland for several years (22). Ruzicka has made use of this reaction of Darzens for preparing certain unsaturated alicyclic ketones to be used in his synthesis of substituted decalins (19, pp. 1157, 1161, 1166).

Aliphatic β -chloroketones have also been described, prepared from isobutylene (13), trimethylethylene (22), ethylene (20), and certain unsaturated cholesterol derivatives (23) with acetyl chloride in the presence of anhydrous aluminium chloride.*

Nenitzescu (16) has found that under certain conditions cyclohexane and many alkyl halides react in the presence of anhydrous aluminium chloride to give a variety of products; the initial dehydrogenation of cyclohexane to cyclohexene is suggested. Hopff (10) showed that many aliphatic and alicyclic hydrocarbons underwent the Friedel-Crafts reaction, but the yields were very small.

β -Chloropropiophenone has hitherto been prepared by the addition of hydrogen chloride to vinyl phenyl ketone (12) and by the action of β -chloropropionyl chloride on benzene in the presence of anhydrous aluminium chloride (1, 5, 7, 9, p. 845). This new procedure might be applied to the production of the homologues of β -chloropropiophenone, except that the necessary acid chlorides are not as readily available, and until the properties of the former were known the method of isolation could not be determined. Accordingly, the writers synthesized nine β -chloropropiophenones, having substituents in the phenyl group, from β -chloropropionic acid; these substances are much

*In a paper that came to hand after this manuscript was completed, the preparation of 1, 4-dichlorobutanone-2 from chloroacetyl chloride and ethylene in the presence of anhydrous aluminium chloride was described (6).

alike in their physical properties. The *p*-phenyl homologue was already known (1) and was the only one to have a much higher melting point, but its purification was very tedious.

Experimental

The apparatus is assembled according to Fig. 1. *A* is a 6–8 litre bottle set at a convenient height above *E*, a 6-litre flat-bottomed flask containing ethylene; the latter is calibrated in 250-cc. units in order to indicate approximately how much gas has been absorbed. Each run requires 12–14 litres. *B*, *C*, and *D* are used in refilling, without disconnecting from the apparatus.

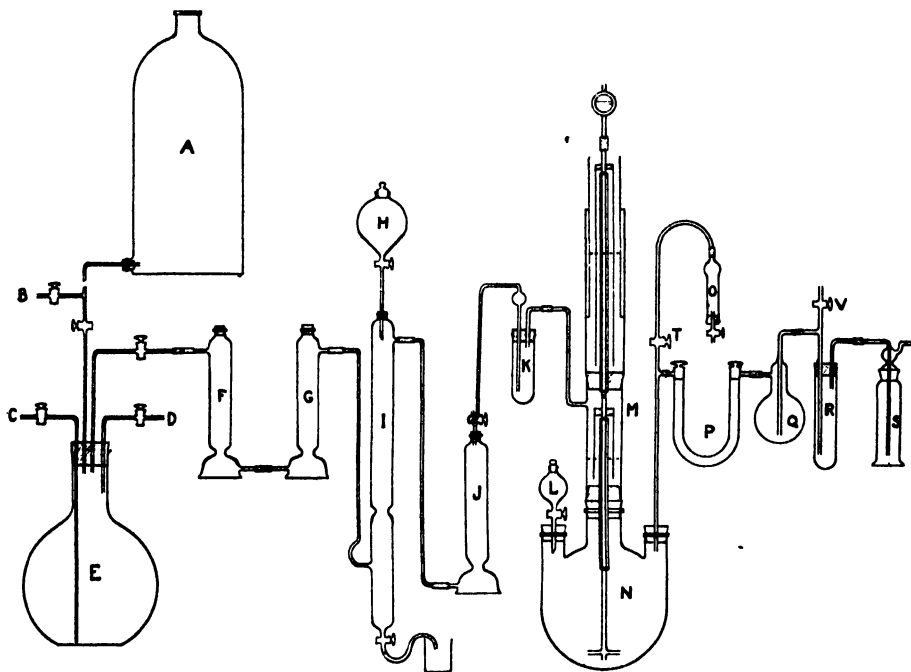


FIG. 1. Diagram of apparatus.

F and *G* are medium-sized drying towers containing anhydrous calcium chloride and small pieces of potassium hydroxide respectively. *I* is an Emmerring tower full of glass beads wet with concentrated sulphuric acid. *J* is a small drying tower containing alternate layers of glass wool and phosphorus pentoxide. *K* and *R* are bubble counters; *K* contains just enough benzoyl chloride to barely seal the end of the inlet tube, but 3 cm. of water are placed in *R*.

The special stirrer *M* (Fig. 2) of Pyrex glass was devised to introduce the ethylene into the solution under slight pressure. The lower part is hollow; a very small hole is blown so that it comes in the open space below the upper stopper. The distance *b*, *b'* is such that it will just pass through the neck of the flask; point *a* is blown out slightly—any constriction is to be avoided.

The tube below *a* is 1 cm. long. The walls of *C* are best constructed of two pieces of Pyrex that fit snugly and can be fastened at *d* by friction tape. This allows the use of a shorter stirrer, since the upper portion can be raised after the motor has been attached; it prevents throwing of mercury and facilitates dismantling. Each part contains mercury up to the dotted line. In operation, the arms of the stirrer are nearly immersed in the solution, and it is rotated at a moderate speed. This is a modification of a stirrer devised by Benning (4).

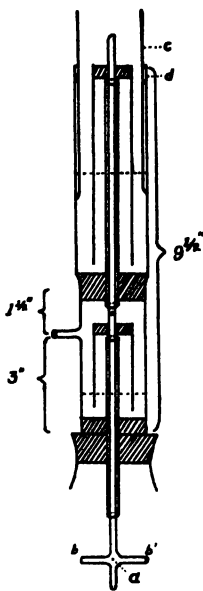


FIG. 2. Stirrer.

N is a one-litre three-necked flask in which the reaction takes place, and is fitted with a small dropping funnel *L* and exit tube connected as shown to *O*, a protective calcium chloride tube, and *P*, a small drying tube filled with potassium hydroxide. The success of the preparation depends upon absolute dryness. *Q* is a trap and *S* a Drechsel bottle holding a 3-cm. layer of bromine covered by water. Rubber tubing and screw pinchcocks may be substituted for most of the glass stopcocks if only one or two runs are to be made.

In flask *N* is placed 60 gm. (0.45 mole) of pulverized anhydrous aluminium chloride* and 100 cc. of ethyl bromide is admitted through *L***. The stirrer is started, and 56.3 gm. (0.4 mole) of technical benzoyl chloride is slowly dropped in from *L* during a half hour†; a soluble double salt results. Ethylene from *E* is now forced in by the water pressure in *A* at such a rate that there is no passing of gas through *R*. It is absorbed very rapidly at first (5 litres in 3.5 hr.). After 7–8 litres have reacted, the rate slackens (40–45 drops of water per minute from *A*)††. After 30–35 hr. a considerable amount of solid separates and the absorption becomes exceedingly slow; when it has practically stopped‡ the reaction is considered complete, although there is still some unused benzoyl chloride. If the operation is continued until the odor of the benzoyl chloride has disappeared, the yield is considerably decreased and much

*The aluminium chloride is the principal source of poor results; the only sure way of determining the suitability of a given lot is to try it out on a quarter-size run. Material that is satisfactory for ordinary Friedel-Crafts reactions may give only small amounts of the chloroketone. In some instances the addition of 1% of anhydrous ferric chloride will make an unsatisfactory grade of aluminium chloride suitable, but ferric chloride alone will not do. Stannic chloride was unsatisfactory.

**If the ethyl bromide is of doubtful quality it should be stored over anhydrous aluminium chloride for 15 min. before use. A slight excess of the latter is used in the preparation to allow for small amounts of water, alcohol, or ether.

†There is a considerable evolution of halogen acid while adding the benzoyl chloride; tube *O* is disconnected, stopcock *T* opened, and the gas absorbed in water—it is not advisable to use a gas trap. The addition of the chloride evolves heat so that it must be added slowly to prevent loss of ethyl bromide.

††The slow absorption is somewhat accelerated by occasionally sweeping out the reaction flask. This is done by opening *V* for a few seconds.

‡To determine the completion of the reaction the flask is swept out and the stopcock *W* is closed; if there is not an appreciable rise of water in the tube of *R* in five minutes the reaction is considered complete.

oily by-product formed. The operation may be interrupted at any time, *i.e.*, need not be continuous. When not in operation it is necessary to lift the stirrer out of the solution and open stopcocks *T* and *V*.

The reaction flask is then removed and its contents poured upon a mixture of 400 gm. of ice and 50 cc. of concentrated hydrochloric acid^{††}. The liquid is filtered from a flocculent precipitate through glass wool. The lower layer is separated and washed twice with 100–150 cc. of water. Flask *N* is rinsed with 250 cc. of benzene, the latter being poured through the glass wool and then used to extract the aqueous upper layer. The combined extracts are dried a half hour with calcium chloride, filtered into a distilling flask and the solvent removed *in vacuo*. It is important to heat the β -chloropropiophenone as little as possible. The solvent is distilled, keeping the temperature of the water bath used as source of heat below 50° C. The distillate is used to extract subsequent runs. The residual yellow-brown oil is poured out into a dish and allowed to solidify.

The crude β -chloropropiophenone is purified by recrystallizing as follows: 250 cc. of petroleum ether[§] is heated to boiling in a suitable wide-mouthed flask under a reflux condenser, the heat removed, and when refluxing has stopped 25 gm. of the crude product added. The solution is boiled several minutes and then filtered through a previously warmed funnel. From the cooled filtrate, yellow flakes, m.p. 52–53° C., separate and are filtered; a second crop is obtained by distilling a part of the solvent. The combined yield is 59–62 gm. (87–92%). It will keep several months in bottles of brown glass. A second crystallization gives an almost white product, m.p. 55–56° C.; the pure ketone melts at 57° C. (12).

The crude solid may be purified by vacuum distillation in 10-gm. lots, if the pressure is kept below 2 mm., followed by recrystallization as above. When β -chloropropiophenone is prepared from β -chloropropionic acid it melts at 49° C., even when white, unless it is put through a treatment with a decolorizing carbon (Darco).

The substituted β -chloropropiophenones were all prepared by the modification (1) of Hale and Britton's method (9, p. 845). The oils remaining after evaporation of the solvent usually solidified when chilled in a freezing mixture. They were all deeply colored and required three or four recrystallizations from petroleum ether (65–80° C.) for purification. Their properties are summarized in Table I.

The products were oily when mesitylene, cumene, pseudo-cumene, cymene, naphthalene, veratrol, and resorcinol dimethyl ether were used. The entering group always took the para position as was shown by identifying the acids

^{††}The ethyl bromide may be distilled off before decomposition and used in subsequent runs, but it is hardly worth while unless molar quantities are being used. In the latter event, a two-litre flask is used. A molar run requires 27–30 litres of ethylene and takes 75–80 hr. The percentage yield is practically the same. The oil remaining after removal of the ethyl bromide is taken up in 700 cc. of benzene and decomposed in the way described above.

[§]Commercial "aeroplane gas" is fractionated and the portion boiling from 65 to 80° C. taken. The 80–100° fraction may also be used provided the temperature is kept below 85° C. during the crystallization. If available, technical cyclohexane is the most satisfactory solvent.

TABLE I

PROPERTIES OF β -CHLOROPROPIOPHENONE HOMOLOGUES

No.	Substituent	Formula	Crystalline form	M.p. ° C.	Yield %	Analysis for Cl**	
						Calcd., %	Found, %
1	F	C_9H_9OFCI	Plates	50	60	19.0	19.0
2	Cl	$C_9H_9OCl_2$	Cubes	48	38	17.4	17.5
3	Br	C_9H_9OCIBr	Leaflets	35	35	14.3	14.2
4	CH_3	$C_{10}H_{11}OCl$	Plates	75	80	21.0	21.2
5	C_2H_5	$C_{11}H_{13}OCl$	Leaflets	55	69	19.4	19.7
6	3, 4-(CH_3) ₂	$C_{11}H_{13}OCl$	Needles	68	60	19.4	19.3
7	CH_3O	$C_{10}H_{11}O_2Cl$	Leaflets	55	60	17.8	18.1
8	C_2H_5O	$C_{11}H_{13}O_2Cl$	Plates	45	82	16.7	16.5
9	C_6H_5O	$C_{15}H_{15}O_2Cl$	Plates	62	99	13.6	13.9
10	C_6H_5	$C_{15}H_{15}OCl$	Needles	112	*		

*See Ref. 1, p. 741.

**The new ketones were analyzed by estimating the chlorine in the beta position, which was easily removed by alcoholic sodium hydroxide.

produced on oxidation. Chromic acid was used for 2, 3, 4, 5 and permanganate for the others. Substituted benzoic acids were produced with all except 4 and 5, which gave terephthalic acid; this was converted into the dimethyl ester by the usual procedure. In all cases except 9, a mixed melting point with an authentic specimen was determined—in the following list, the observed melting point is given first, then the melting point of the mixture with the reference compounds: (1) 182, 182; (2) 233, 235; (3) 251, 248; (4 and 5) 140, 140; (6) 160, 159; (7) 180, 182; (8) 190, 194; (9) 158†; (10) 215, 218° C.

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†The value given in the literature is 159.5° C. (11).

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**A list of earlier references is given in this paper.*

THE IONIZATION CONSTANTS OF *p*-NITROPHENYLACETIC AND PHENYLMALONIC ACIDS¹

BY STEWARD BASTERFIELD² AND JAMES W. TOMECKO³

Abstract

The ionization constants of *p*-nitrophenylacetic and phenylmalonic acids have been determined from conductivity data. The value of K for *p*-nitrophenylacetic acid at 25° C. is 1.04×10^{-4} , about twice that of phenylacetic acid. The nitro group in the nucleus has not as powerful an effect on the ionization when the COOH group is in the side chain as it has when both nitro group and COOH are in the nucleus. K for *p*-nitrobenzoic acid is six times as great as K for benzoic acid. K for phenylmalonic acid is 2.77×10^{-3} as compared with 1.6×10^{-3} for malonic acid.

In connection with studies on the urethanes of *p*-nitrophenylacetic and phenylmalonic acids carried on in this laboratory, it was desired to know the values of the ionization constants of these acids, and a search of the Landolt-Börnstein Tables and the International Critical Tables failed to reveal them. Although a reference is made by Meldrum (1) to a measurement of the constant of phenylmalonic acid by Walden, a search failed to locate it, and no value seems to have been recorded officially. The present study was undertaken in consequence.

The ionization constant of *p*-nitrophenylacetic acid was determined by the standard conductance method. The acid is comparatively weak, and in order therefore to calculate Λ_{∞} for the acid, the conductance of the sodium salt had to be determined. The conductance of a series of solutions of the salt was measured and the values of equivalent conductances plotted against the square roots of the concentrations and Λ_{∞} obtained by extrapolation. Λ_{∞} for the acid was then calculated by the principle of Kohlrausch. The conductance of a series of solutions of the acid was next determined, and values of Λ inserted in Ostwald's dilution formula to obtain K .

In the study of phenylmalonic acid, the ionization constant for the first hydrogen only was determined. To evaluate Λ_{∞} for the acid the conductance of the monosodium salt had to be measured. The usual method of measuring the conductances of a series of solutions, and extrapolating to find Λ_{∞} could not be used, since at high dilutions the second hydrogen becomes dissociated. Meldrum (1) in a study of a series of alkylmalonic acids found that at a dilution of 1024 litres, Λ for the monosodium salt is practically identical with Λ_{∞} calculated from two values of Λ at different concentrations by means of the relation:—

$$\frac{\Lambda_1^2 C_1}{\Lambda_{\infty}(\Lambda_{\infty} - \Lambda_1)} = \frac{\Lambda_2^2 C_2}{\Lambda_{\infty}(\Lambda_{\infty} - \Lambda_2)} = K.$$

¹ Manuscript received March 6, 1933.

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² Professor of Organic Chemistry, University of Saskatchewan.

³ Graduate student, University of Saskatchewan.

Following this method therefore, the value of Λ for a 0.001 *M* solution of the sodium salt was taken as Λ_{∞} for the salt with the sodium ion completely dissociated. The value of Λ_{∞} for the acid was then calculated. Values of Λ for a series of solutions of the acid were measured and K calculated by the Ostwald formula as before.

The values of K obtained for the two acids are given below together with values of related acids for comparison. It will be noted that the introduction of the nitro group into phenylacetic acid doubles the value of the ionization constant. The effect of the NO_2 group here is not so great as in *p*-nitrobenzoic acid, where both the nitro group and the COOH are attached to the nucleus. K for *p*-nitrobenzoic acid is about six times as great as for benzoic acid. The introduction of the nitro group into the *p*- position in phenoxyacetic acid increases the ionization constant to about the same extent as does its introduction into phenylacetic acid.

The introduction of the phenyl group into acetic acid increases the ionization constant threefold, while a similar introduction into the much stronger malonic acid increases the value of K by barely three-fourths.

TABLE I
VALUES OF K FOR VARIOUS ACIDS

Acid	K (at 25° C.)	Acid	K (at 25° C.)
Acetic	1.81×10^{-5}	Malonic	1.61×10^{-3}
Phenylacetic	5.45×10^{-5}	Phenylmalonic	2.77×10^{-3}
<i>p</i> -Nitrophenylacetic	1.04×10^{-4}	Benzoic	6.6×10^{-5}
Phenoxy acetic	7.40×10^{-4}	<i>p</i> -Nitrobenzoic	4.0×10^{-4}
<i>p</i> -Nitrophenoxy acetic	1.50×10^{-3}		(at 99° C.)

Experimental

p-Nitrophenylacetic Acid and its Sodium Salt

p-Nitrophenylacetic acid was recrystallized from hot water until its melting point was constant (152° C.). The sodium salt was prepared by treating an aqueous solution of the acid with a slight excess of sodium hydroxide. The solution was brought to the neutral point with a few drops of hydrochloric acid, and evaporated to dryness on the steam bath. The residue was extracted with absolute alcohol, and the sodium *p*-nitrophenylacetate precipitated from the alcohol solution by the addition of anhydrous ether. The salt was collected, washed with ether and dried, and reprecipitated several times from alcoholic solution.

A series of solutions of the salt in conductivity water was made gravimetrically and the equivalent conductances determined in the usual way at 25° C. Λ_{∞} for the salt was obtained by extrapolation of the curve of equivalent conductance plotted against the square root of the concentration.

The equivalent conductances of a series of solutions of the acid were determined in similar fashion, and K calculated from Ostwald's dilution formula. The results are tabulated below.

TABLE II

CONDUCTANCE OF SODIUM *p*-NITROPHENYLACETATE.
CELL CONSTANT 1.0227

Molar concentration	Resistance	Equiv. conductance
0.01	1008	101.46
.005	1967	104.00
.0025	3815	107.24
.001	9230	110.70
.000		113.00

Λ_{∞} (salt), 113 Λ_{∞} (Na^+), 48.82 Λ_{∞} (anion), 64.18
 Λ_{∞} (H^+), 350 $\therefore \Lambda_{\infty}$ (acid) = 64.18 + 350 = 414.18

TABLE III

CONDUCTANCE OF *p*-NITROPHENYL-
ACETIC ACID

Molar concentration	Resistance	Equiv. conductance	$K \times 10^4$
0.01	2479	41.43	1.117
.005	3585	57.06	1.101
.001	9185	113.70	1.037
.0005	14600	143.14	.912

Mean value of K , 1.041×10^{-4}

Phenylmalonic Acid and its Monosodium Salt

Phenylmalonic acid prepared from the hydrolysis of methyl phenylmalonate, was purified by five recrystallizations from a mixture of ether and ligroin. It melted at 152–153° C. An aqueous solution of the acid was treated with rather less than one equivalent of sodium hydroxide. The solution was evaporated to dryness at a little above room temperature, and the slight excess of phenylmalonic acid extracted from the residue with ether. The sodium salt was washed repeatedly with dry ether. It melted at 184–188° C. with decomposition. A 0.001 *M* solution was prepared gravimetrically and the equivalent conductance determined. This value was taken as Λ_{∞} for the salt, with Na^+ ion completely dissociated. Cell constant, 1.0105. Molar concentration, 0.001. Resistance, 9090. Equivalent conductance, 111.14. Λ_{∞} (salt), 111.14. Λ_{∞} (Na^+), 48.82. Λ_{∞} (anion), 62.32. Λ_{∞} (H^+), 350.00. $\therefore \Lambda_{\infty}$ (acid) = 62.32 + 350 = 412.32.

TABLE IV

CONDUCTANCE OF PHENYLMALONIC ACID

Molar concentration	Resistance	Equiv. conductance	$K \times 10^3$
0.01	597.5	169.10	2.85
.008	691.0	182.75	2.82
.005	956.0	211.40	2.70
.004	1120.00	225.20	2.70

Mean value K , 2.77×10^{-3}

Reference

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STUDIES IN URETHANES

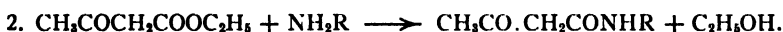
VI. ACYL MONOURETHANES AND THEIR REACTIONS WITH AMMONIA AND AMINES¹BY STEWARD BASTERFIELD² AND MARGARET E. GREIG³

Abstract

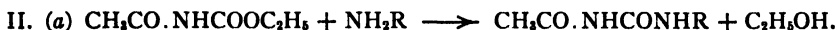
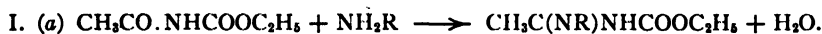
The study of the action of ammonia and amines on acyl urethanes has been extended to urethanes of monobasic acids in order to determine whether the rule tentatively formulated with respect to the diurethanes (2, p. 361) holds for the monourethanes. The results reported here provide considerable evidence that the rule is broadly true, the tendency for formation of acid amides or ureas varying with the strengths of the acids and bases involved.

The results of the study of the action of ammonia and primary amines on acyl-diurethanes as reported in previous papers (2, 3), suggested the extension of the investigation to the behavior of acyl monourethanes with the ammonia bases. Very little attention seems to have been paid to this type of reaction. Werner (5) has shown that strong aqueous ammonia at room temperature has no action on urethane and at 100° C. causes only a 10% conversion of urethane to urea in 20 hr., on account of the reversibility of the reaction, though it is also a well-known fact that urethane may be completely transformed into diphenyl-urea by heating with aniline at 150° C. for an hour.

The literature showed only one account of the action of ammonia bases on acyl monourethanes, namely, a study of acetyl-urethane by Young and Clark (6). They undertook the investigation of these reactions on the basis of a supposed analogy with the action of ammonia bases on acetoacetic ester. The latter forms two series of derivatives, the reaction being represented by the equations:



Acetyl-urethane, if it behaved similarly should yield carbethoxy-amidines, and acetyl-ureas as follows:



Using ammonia and aniline as bases, they found that acetyl-urea and acetylphenyl-urea were generally formed, and under certain conditions, acetamide and acetanilide. In no case were they able to isolate an amidine, although they explain the appearance of acetamide and acetanilide as being due to the hydrolysis of this hypothetical compound. It seems quite unnecessary to invoke the aid of any such hypothesis to interpret the results of the

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Contribution from the Chemical Laboratories, University of Saskatchewan, Saskatoon, Canada. This paper is based on a thesis presented by Margaret E. Greig in partial fulfilment of the requirements for the degree of M.A. at the University of Saskatchewan.

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reaction, and it would doubtless not have arisen except for the superficial structural resemblance of acetyl-urethane to acetoacetic ester. The carbonyl group of acetyl-urethane is not a ketone carbonyl, and would not be likely to function in the same way as the carbonyl group of the keto-ester.

To gain more insight into the mechanism of this type of reaction and at the same time to observe the effect of the presence of substituent groups, the following compounds were chosen as suitable examples for study: phenyl-acetyl and *p*-nitrophenylacetyl-urethanes; chlor- and bromacetyl-urethanes; benzoyl-, *p*- and *m*-nitrobenzoyl-urethanes. All these urethanes were readily prepared with the exception of the last named. *m*-Nitrobenzoyl chloride and urethane under a variety of conditions gave *m*-nitrobenzoyl-allophanic ester, *m*-nitrobenzoyl-carbethoxy-biuret, and another product probably α - β -di-(*m*-nitro-benzoyl)-allophanic ester, but no *m*-nitrobenzoyl-urethane. Attention was confined therefore to a comparison of results with benzoyl and *p*-nitrobenzoyl-urethanes.

The details of the experiments performed are given below but the results may be summarized here. Chlor- and bromacetyl-urethanes are decomposed at room temperature by concentrated aqueous ammonia into chlor- and bromacetamides. No ureas were obtained. With aniline at temperatures ranging from 100° to 190° C., the main products from both these urethanes were the same, *viz.*, α -phenylglycyl- β -phenyl-urea, the halogen having been replaced by the phenylamino group. The effect of the halogen on the course of the ammonolysis at the higher temperature was thus completely offset.

Phenylacetyl-urethane gave with aqueous ammonia and aqueous ethylamine at room temperature, phenyl-acetamide, and *N*-ethyl-phenylacetamide, respectively, as the main products, together with small quantities of the ureas. With aniline at 100° and 150° C., α -phenyl- β -phenylacetyl-urea was formed; at the latter temperature the yield was 92% of the theoretical. No phenyl-acetanilide or diphenyl-urea were detected at these temperatures after two hours' heating. At 190° C. phenylacetanilide and diphenyl-urea were obtained almost quantitatively after two hours' heating. The phenylacetyl-urethane thus behaves very similarly to the urethanes of succinic, glutaric, and adipic acids in giving as the first product a urea, which undergoes decomposition at the higher temperatures to yield the anilide (2).

p-Nitrophenylacetyl-urethane showed very similar results except that the urea obtained by the action of aniline was less stable and underwent appreciable decomposition at 150° C. during two hours' heating. *p*-Nitrophenylacetanilide and diphenyl-urea were always obtained at this temperature, the amounts representing about 12% decomposition of the urea. Reference to the table of ionization constants (1, p. 448) shows that *p*-nitrophenylacetic acid has a constant about twice as large as that of phenylacetic acid.

The comparison of benzoyl- and *p*-nitrobenzoyl-urethanes showed marked differences in their behavior towards ammonia and amines. Benzoyl-urethane with strong aqueous ammonia at room temperature gave mainly benzoyl-urea,

and with ethylamine under the same conditions benzamide and an oil which was apparently N-ethylethyl-urethane. This decomposition is unusual and resembles that of carbonyl-diurethane by ethylamine (3). With aniline at 150° C. a quantitative yield of α -benzoyl- β -phenyl-urea was obtained, while at 190° C. benzanilide and diphenyl-urea were obtained, representing complete decomposition of the urea.

p-Nitrobenzoyl-urethane with ammonia gave *p*-nitrobenzamide. No urea was detected. With ethylamine the main product was N-ethyl-*p*-nitrobenzamide, a result quite in accordance with those obtained with phenylacetyl- and *p*-nitrophenylacetyl-urethanes. With aniline at temperatures from 100° to 190° C. for a period of two hours, the same products were obtained, *viz.*, *p*-nitrobenzanilide and diphenyl-urea, the yields being greater, of course, at higher temperatures. No intermediate formation of a urea was observed. Observations at lower temperatures were not made but it is evident that, if any urea is formed, it must be relatively unstable even at 100° C. and must disappear quickly. The ionization constant of *p*-nitrobenzoic acid is about six times that of benzoic acid (1, Table I).

It seems evident from these results that the mode of reaction of these urethanes with bases is definitely influenced by the nature of the acyl radical present as well as by that of the base, and lends further support to the qualitative generalization arrived at in previous studies, namely, that with a given amine the tendency to amide as compared with urea formation is more marked with urethanes containing acyl radicals of stronger acids, while with a given urethane the amide formation occurs more readily with the stronger bases. It is not suggested that this is the only factor which determines the mechanism of reaction, but that it is a prominent one seems beyond question. There are doubtless other factors affecting the stability of the ureas that are formed as intermediate products in many of these reactions, and it is hoped that studies being carried on in this laboratory will throw light on these.

It is an interesting fact that while urethane itself undergoes little or no change when left in contact with strong ammonia at room temperature, the acyl urethanes are converted into ureas or amides to a considerable extent. If Werner's idea of the mechanism of urea formation from urethane is correct, it would imply that the acyl urethanes are appreciably dissociated at room temperature into an "acyl isocyanide" and alcohol, and that the formation of acyl ureas by this reaction is not markedly reversible.

One other feature of these reactions is worthy of note. Although aqueous solutions of both ammonia and ethylamine behave as bases of the water system, and to some extent bring about hydrolysis of the compounds studied here, they display ammonolytic properties much more conspicuously than do alcoholic solutions of the same bases. In other words water appears to catalyze the process of ammonolysis. In alcoholic solutions of the bases, alcoholysis of the urethanes occurred to some extent, with the production of esters. These points are best illustrated in the sections on phenylacetyl- and *p*-nitrophenylacetyl-urethanes in the experimental part.

Experimental

PREPARATION OF ACYL MONOURETHANES

1. *Chloracetyl-urethane*, $\text{ClCH}_2\text{CO.NHCOOC}_2\text{H}_5$. Equimolecular proportions of chloracetyl chloride and urethane were heated together on a water bath until the evolution of hydrogen chloride had ceased. The product was recrystallized from alcohol; m.p., 129°C .

2. *Bromacetyl-urethane*. $\text{BrCH}_2\text{CO.NHCOOC}_2\text{H}_5$. This was prepared in a similar manner to the above; m.p., 119°C . The yields in both cases were low, about 35% of the theoretical. Allophanic ester was a by-product.

3. *Phenylacetyl-urethane*, $\text{C}_6\text{H}_5\text{CH}_2.\text{CONHCOOC}_2\text{H}_5$. This urethane was prepared by the method of Basterfield, Woods, and Whelen (3).

4. *p-Nitrophenylacetyl-urethane*, $\text{NO}_2\text{C}_6\text{H}_4\text{CH}_2\text{CO.NHCOOC}_2\text{H}_5$. In the first attempt to prepare this urethane, *p*-nitrophenylacetic acid, and urethane in equimolecular proportions were heated with an excess of phosphorus oxychloride at 90°C . for two hours with constant stirring. A small yield of the urethane resulted, and a considerable amount of a compound probably *p*-nitrophenylacetyl-allophanic ester: m.p., 190°C . Calcd. N, 14.23. Found: 14.8%. Better results were obtained by first preparing the acid chloride.

p-Nitrophenylacetic acid was heated with an excess of thionyl chloride for 10–12 hr. at 60°C . On cooling the mixture the acid chloride crystallized out. After washing with ligroin it melted at 49° – 50°C . and was obtained in quantitative yield.

When the acid chloride was heated with urethane at 100°C . for about ten hours, the acyl urethane was obtained in 90% yield. It was recrystallized from alcohol; m.p. 175°C . Calcd. for $\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}_5$; N, 11.20. Found: N, 10.97, 11.30%.

5. *Benzoyl-urethane*, $\text{C}_6\text{H}_5\text{CO.NHCOOC}_2\text{H}_5$. When benzoyl chloride and urethane were heated together without a solvent at 100°C . only benzoylallophanic ester was obtained (m.p. 175°C .). Using the method of Pechmann and Vanino (4), in which pyridine is introduced into the reaction mixture, a mixture of acyl urethane and allophanic ester was obtained; yield of benzoyl-urethane, 42% (m.p., 111°C .).

6. *p-Nitrobenzoyl-urethane*, $\text{NO}_2.\text{C}_6\text{H}_4.\text{CO.NHCOOC}_2\text{H}_5$. Equimolecular proportions of *p*-nitrobenzoyl chloride and urethane in benzenewere refluxed until no further evolution of hydrogen chloride took place; yield, 60%. *p*-Nitrobenzoyl-allophanic ester was also formed in varying amounts. The great instability of the urethane renders its purification difficult; m.p., 152°C . Calcd. for $\text{C}_{10}\text{H}_{10}\text{N}_2\text{O}_5$; N, 11.7. Found: N, 11.3, 11.3%.

It is decomposed by heating in alcohol solution, with the production of *p*-nitrobenzoyl-allophanic ester; m.p., 194°C . Calcd. for $\text{C}_{11}\text{H}_{11}\text{N}_3\text{O}_6$; N, 14.93. Found: N, 14.57, 14.79%.

7. *m-Nitrobenzoyl-urethane*. *m*-Nitrobenzoyl chloride and urethane in equimolecular proportions were heated on a water bath without a solvent for

several days. A substance melting at 128° C. was obtained. It was apparently *m*-nitrobenzoyl-allophanic ester, $\text{NO}_2 \cdot \text{C}_6\text{H}_4 \cdot \text{CONHCONHCOOC}_2\text{H}_5$. Calcd. for $\text{C}_{11}\text{H}_{11}\text{N}_3\text{O}_6$; N, 14.93. Found: N, 14.90, 14.90%.

During the recrystallization of this compound from alcohol, it was partly converted into a substance melting at 140° C., probably *m*-nitrobenzoyl-carbethoxy-biuret, $\text{NO}_2 \cdot \text{C}_6\text{H}_4 \cdot \text{CONH} \cdot \text{CONH} \cdot \text{CONH} \cdot \text{COOC}_2\text{H}_5$. Calcd. for $\text{C}_{12}\text{H}_{12}\text{N}_4\text{O}_7$; N, 17.2. Found: N, 17.49, 17.41%.

When the acid chloride and urethane were refluxed in benzene still another compound was obtained, melting after recrystallization from alcohol; m.p., 164° C. Found: N, 14.2%. The only substance that seems to have the required nitrogen content is α - β -di-(*m*-nitrobenzoyl)-allophanic ester, $\text{NO}_2 \cdot \text{C}_6\text{H}_4 \cdot \text{CO} \cdot \text{NH} \cdot \text{CO} \cdot \text{N}(\text{COC}_6\text{H}_4\text{NO}_2) \cdot \text{COOC}_2\text{H}_5$. Calcd. N, 14.0%.

No *m*-nitrobenzoyl-urethane was obtained and lack of time prevented any further investigation of the above compounds.

ACTION OF AMMONIA AND AMINES ON ACYL MONOURETHANE

1. Chloracetyl-urethane

(a) *Action of ammonia.* The urethane was allowed to stand in contact with alcoholic ammonia at room temperature for one week. A 92% yield of chloracetamide; m.p. 116° C. was obtained.

(b) *Action of aniline at 100°, 150°, and 190° C.* Chloracetyl-urethane (2 gm.) was treated with five molecular proportions of aniline for two hours at these temperatures. The semisolid mass was washed with ether and recrystallized from alcohol. The same product was obtained in all the experiments: maximum yield, 1½ gm.; m.p., 195° C. Found: N, 15.57%. No halogen was present. The compound was apparently α -phenyl-glycyl- β -phenyl-urea, $\text{C}_6\text{H}_5\text{NHCH}_2\text{CO} \cdot \text{NHCO} \cdot \text{NHC}_6\text{H}_5$ (N, 15.61%). It was evident that at these temperatures the halogen of the urethane reacted with aniline. This is supported by the fact that bromacetyl-urethane was found to yield the same product.

2. Phenylacetyl-urethane

(a) *Action of ammonia.* Phenylacetyl-urethane (2 gm.) was allowed to stand in contact with strong ammonia solution for one week. The solid residue, after evaporation of the ammonia at room temperature, melted at 145° C. The substance was recrystallized from alcohol, and separated into two parts, the more insoluble being phenylacetyl-urea (m.p. 209° C.). Yield, .6 gm. The other fraction consisted of phenyl-acetamide: m.p., 152° C.; yield, 76%.

In alcoholic ammonia solution the reaction was very slow, the bulk of the urethane being unchanged after a week. A small quantity of phenylacetamide was obtained.

(b) *Action of ethylamine.* Phenylacetyl-urethane (2 gm.) was mixed with excess of aqueous ethylamine (17%), and allowed to stand for one week at room temperature. The liquid was evaporated and a waxy solid remained.

From this by extraction with ligroin was obtained mainly N-ethyl-phenyl-acetamide, $C_6H_5CH_2CO.NHC_2H_5$, m.p. $75^\circ C$. Calcd. for $C_{10}H_{13}NO$; N, 8.58. Found: N, 8.44%. A very small amount of a substance melting at $156^\circ C$., probably α -phenylacetyl- β -ethyl-urea, $C_6H_5CH_2CONHCONHC_2H_5$, was also isolated. Calcd. for $C_{11}H_{14}N_2O_2$; N, 13.5. Found: N, 13.0%.

(c) *Action of aniline.* (i) At $100^\circ C$. Phenylacetyl-urethane was slowly converted by aniline into α -phenyl- β -phenylacetyl-urea, $C_6H_5NHCO.NH.COCH_2C_6H_5$; m.p., $165^\circ C$.; yield, 66%, after three hours' heating.

(ii) At $150^\circ C$. Two hours' heating (one molecular proportion of urethane to five of aniline) gave a 92% yield of α -phenyl- β -phenylacetyl-urea. There was no diphenyl-urea or phenyl-acetanilide found at this temperature in any experiment.

(iii) At $190^\circ C$. Two hours' heating of the usual reaction mixture brought about an almost complete transformation of the urethane into phenyl-acetanilide and diphenyl-urea. That the decomposition involved the initial formation of the phenylacetylphenyl-urea was shown by heating the latter obtained at $150^\circ C$., with additional aniline to $170^\circ C$., when it was rapidly changed to diphenyl-urea and phenyl-acetanilide. These two compounds were identified by means of mixed melting point determinations with known samples.

3. *p*-Nitrophenylacetyl-urethane

Action of aqueous ammonia. The urethane was allowed to stand in concentrated aqueous ammonia solution for one week. A crystalline solid separated; m.p., $181^\circ C$. This was resolved by recrystallization from hot alcohol into two products. The first melted at $235^\circ C$. and proved to be *p*-nitrophenylacetyl-urea, $NO_2C_6H_4CH_2CO.NH.CONH_2$. Calcd. for $C_9H_9N_3O_4$; N, 18.83. Found: N, 19.04%.

The second was *p*-nitrophenyl-acetamide, $NO_2C_6H_4CH_2CONH_2$; m.p., $191^\circ C$.; yield, 85%. Calcd. for $C_8H_8N_2O_3$; N, 15.5. Found: N, 15.5%.

Action of alcoholic ammonia. The urethane was left in contact with weak alcoholic ammonia (6%) for one week. A 28% yield of *p*-nitrophenyl-acetamide was obtained, together with a small amount of ethyl *p*-nitrophenyl-acetate, m.p. $69^\circ C$. Calcd. for $C_{10}H_{11}NO_4$; N, 6.69. Found: N, 6.49, 6.53%. The formation of the ester is interesting as indicating an alcoholysis of the urethane, as well as an ammonolysis. A good part of the urethane was recovered unchanged.

Action of aqueous ethylamine. A sample of the urethane was heated with an excess of aqueous ethylamine (17%) and left for a week at room temperature. The mixture darkened and a solid separated (m.p., $155^\circ C$.). Recrystallization did not change the melting point. The substance was N-ethyl-*p*-nitrophenyl-acetamide ($NO_2C_6H_4CH_2CONHC_2H_5$); yield, 60%. Calcd. for $C_{10}H_{13}N_2O_3$; N, 13.46. Found: N, 13.23, 13.35%.

Action of alcoholic ethylamine. For comparison with the action of alcoholic ammonia an experiment was made using alcoholic ethylamine (6%). In one

week the only product obtained was ethyl *p*-nitrophenyl-acetate in 37% yield. There was no amide formation. When the experiment was carried out over intervals of two or three days, there was no evidence of amide formation. Varying amounts of the ester alone were isolated.

It is evident that ammonolysis by ethylamine is catalyzed by water, since amide formation took place in the aqueous solution and not in the alcoholic.

Action of aniline. (i) *At 100° C.* There was no appreciable reaction after two hours' heating. This is in rather striking contrast to the behavior of phenylacetyl urethane.

(ii) *At 150° C.* Experiments at this temperature were repeated many times, and the yields of the different products carefully checked. Diphenyl-urea and *p*-nitrophenyl-acetanilide were always obtained in small quantity in two hours' heating, along with *p*-nitrophenylacetyl-phenyl-urea. These results definitely point to the latter compound being less stable at this temperature than the phenylacetyl-phenyl-urea. The following is a typical experiment: the urethane (4 gm.) was heated with aniline (7.4 gm.) for two hours in an oil bath at 150° C. The semisolid mass was washed with ether. The ether washings contained both diphenyl-urea (m.p. 236° C.) and *p*-nitrophenyl-acetanilide, m.p. 198° C. Calcd. for $C_{14}H_{12}N_2O_3$; N, 10.90. Found: N, 10.63, 10.84%. The residue after washing with ether was recrystallized from hot alcohol. The greater portion consisted of α -*p*-nitrophenylacetyl- β -phenyl urea, $NO_2C_6H_4.CH_2CO.NH.CO.NHC_6H_5$, m.p. 220° C. Calcd. for $C_{15}H_{13}N_2O_3$; N, 14.05. Found: N, 13.73%. A further quantity of *p*-nitrophenyl-acetanilide was also obtained. Yields: α -*p*-nitrophenylacetyl- β -phenyl-urea, 4.0 gm. (80%); *p*-nitrophenyl-acetanilide, 0.5 gm. (12%); diphenyl-urea, 0.4 gm. (12%).

(iii) *At 190° C.* There was complete decomposition of the urethane into *p*-nitrophenyl-acetanilide and diphenyl-urea.

4. Benzoyl-urethane

Action of ammonia. Benzoyl-urethane was treated with concentrated aqueous ammonia for three days at room temperature. An insoluble crystalline substance separated rapidly. After recrystallization from alcohol it melted at 215° C. It was identified as benzoyl-urea, $C_6H_5CONHCONH_2$. Yield, 63%.

Action of ethylamine. Benzoyl-urethane was left in contact with aqueous ethylamine (17%) for one week at room temperature. The clear solution was evaporated at the same temperature, and a residue of oil and crystals obtained. The oil was taken up in chloroform and the extract distilled. After the chloroform had evaporated, the oil distilled mainly at 175° C. It was apparently N-ethyl-urethane, $C_2H_5NH.COOC_2H_5$, since it yielded ethylamine on being hydrolyzed with sodium hydroxide. The crystalline substance melted, after recrystallization from alcohol, at 129° C. and was identified by a mixed melting point determination as benzamide.

This decomposition is similar to that of carbonyl-diurethane by ethylamine (3), ammonolysis removing the carbethoxy group and not the whole urethane group.

Action of aniline. (i) At 100° and 150° C. Benzoyl-urethane was heated with five molecular proportions of aniline for two hours. At 100° C. an insignificant amount of α -benzoyl- β -phenyl-urea, (m.p. 206° C.) $C_6H_5CO.NH.CO.NHC_6H_5$, was obtained, but at 150° C. the yield was 98%. Calcd. for $C_{14}H_{13}N_2O_2$: N, 11.60. Found: N, 11.39%.

(ii) At 190° C. After two hours' heating, diphenyl-urea and benzanilide, m.p. 160° C., were obtained in approximately 95% yield. The benzanilide was identified by a mixed melting point determination.

5. *p*-Nitrobenzoyl-urethane

Action of ammonia. The main product after action for one week was *p*-nitrobenzamide, m.p. 189° C. Calcd. for $C_7H_6N_2O_3$: N, 16.0. Found: N, 15.92, 15.99%.

Action of ethylamine. After one week's action an insoluble product was filtered off, and recrystallized from alcohol. It was apparently *N*-ethyl-*p*-nitrobenzamide, $NO_2.C_6H_4.CONHC_2H_5$; m.p., 149° C. Calcd. for $C_9H_{10}N_2O_3$: N, 14.43. Found: N, 14.09%.

Action of aniline at 100°, 120°, 150° and 190° C. At each of these temperatures after two hours' heating the same products were obtained, viz., diphenyl-urea, and *p*-nitrobenzanilide, m.p. 212° C. No intermediate formation of *p*-nitrobenzoylphenyl-urea was detected. It is evident that the NO_2 group has a very marked effect on the mode of reaction of the urethane, the tendency to amide formation being so strong that either the urea formation is completely eliminated, or the intermediate urea is so unstable that it is immediately decomposed even at 100° C. Although the second alternative seems highly improbable, it has not been definitely ruled out by experiment.

Acknowledgment

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STUDIES IN ISOUREAS AND ISOUREIDES

III. THE IONIZATION CONSTANTS OF SOME ISOUREAS¹BY STEWARD BASTERFIELD² AND J. W. TOMECKO³

Abstract

The ionization constants of seven isoureas have been measured. The values for simple alkyl isoureas increase definitely from methyl to *n*-propyl, the *n*-butyl-isourea showing no further increase. Isobutyl-isourea has a decidedly greater constant than the *n*-butyl compound. Introduction of the phenyl group into ethyl-isourea lowers the value of the constant as would be expected. The presence of a double bond in the radical also produces a similar effect as shown by a comparison of the values for *n*-propyl- and allyl-isoureas. Cyclohexyl-isourea has a constant slightly higher than those of *n*-propyl and *n*-butyl-isoureas. Some comparison with a series of primary amines is made.

Methyl- and ethyl-isoureas were first prepared by Stieglitz and his collaborators (3, 4), and the ionization constants of the two bases were measured by Bruce (3). The values were considerably larger than that of ammonia, the ethyl compound being a rather stronger base than the methyl (See Table I).

Recently a series of new isoureas has been prepared in this laboratory (1, 2) and the present study deals with the determination of the ionization constants of some of these by the conductance method.

Since the free isoureas are too feebly ionized for the direct determination of values of Λ_{∞} , the latter were determined by the principle of Kohlrausch from Λ_{∞} for salts of the bases. With the exception of cyclohexyl-isourea, of which the hydrochloride was prepared, the bases were converted into the salicylates which are well crystallized salts of good conducting power.*

To obtain Λ_{∞} for these, the equivalent conductances were plotted against the square root of the concentrations and extrapolated to infinite dilution. Λ_{∞} for the isourea ions was then calculated from Kohlrausch's law, and hence Λ_{∞} for the bases. Values of Λ for a series of solutions of the free bases were measured and substituted in Ostwald's dilution formula,

$$\frac{\Lambda^2 \cdot c}{\Lambda_{\infty} (\Lambda_{\infty} - \Lambda)} = k.$$

In Table I are given the ionization constants of the isoureas, and for comparison the constants of primary amines containing the same radicals, so far as these are available.

¹ Manuscript received March 6, 1933.

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*The hydrochlorides of the isoureas which are first obtained in the preparation of these compounds, are mostly hygroscopic semicrystalline masses very difficult to purify (1, p. 264). A paper on the preparation and identification of some new isoureas will be published shortly.

TABLE I

IONIZATION CONSTANTS OF ISOUREAS AND PRIMARY AMINES

Isoureas	$K \times 10^4$	Primary amines**	$K \times 10^4$	Isoureas	$K \times 10^4$	Primary amines**	$K \times 10^4$
Methyl*	0.60	Methyl	5.0	Allyl	0.50	Allyl	0.49
Ethyl*	1.08	Ethyl	5.6	<i>n</i> -Butyl	1.40	(Sec.-butyl)	(4.4)
		Phenylmethyl	0.2	Isobutyl	2.00	Isobutyl	2.6
Phenylethyl	0.62						3.1
<i>n</i> -Propyl	1.43	<i>n</i> -Propyl	{ 3.9	Isoamyl	1.30	Isoamyl	5.0
			4.7	Cyclohexyl	1.56		4.0

*Measurements by Bruce (3, p. 457).

**Values for amines are taken from *International Critical Tables and Landolt-Börnstein Tabellen*. Where two values are given for an amine the higher value is that given by the latter authority.

It is apparent that, on the whole, the aliphatic primary amines are considerably stronger bases than the alkyl isoureas. The effect of the introduction of the phenyl radical or of a double bond into the alkyl radical in diminishing the ionization constant is, however, very much greater in the amines than in the isoureas. So much so that phenylmethylamine is actually a weaker base than phenylethyl-isourea, while allyl-isourea and allylamine show almost identical values for their ionization constants. The ionization constants of phenylethylamine, *n*-butylamine, and cyclohexylamine are not recorded in the literature.

Experimental

The salts of the isoureas were crystallized several times from alcohol and series of solutions of different concentrations were made gravimetrically. Values of Λ_∞ for the different salts were determined as indicated in the preceding section. The conductance curves for the salts examined are shown in Fig. 1.

The free isoureas were prepared by treating the salts, suspended in moist ether, with four molecular proportions of powdered potassium hydroxide. The ethereal solutions of the bases were filtered and dried over anhydrous sodium sulphate. After removal of the ether, the bases were dried as completely as possible in a vacuum over concentrated sulphuric acid and powdered

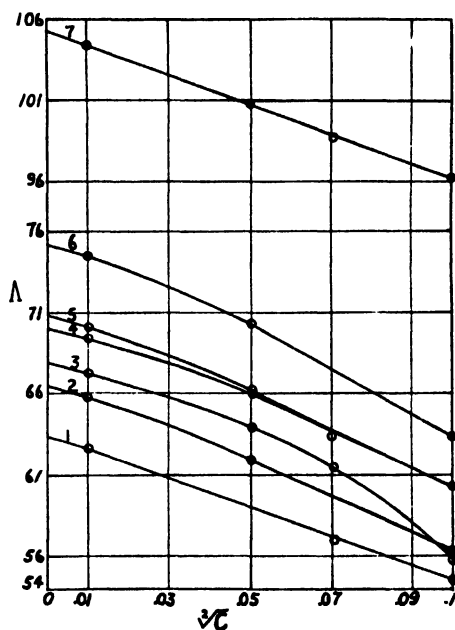


FIG. 1. Conductance of isourea salts. 1, Phenylethyl-isourea salicylate. 2, *n*-Butyl-isourea salicylate. 3, *n*-Propyl-isourea salicylate. 4, Isobutyl-isourea salicylate. 5, Allyl-isourea salicylate. 6, Isoamyl-isourea salicylate. 7, Cyclohexyl-isourea hydrochloride.

potassium hydroxide. With the exception of cyclohexyl-isourea, which was obtained as a solid of low melting point, the bases were oils. Previous experience had shown that the free isoureas are too sensitive to heat, to be purified even by vacuum distillation, so that no other purification was possible except what was involved in the repeated recrystallization of the salts, and the use of pure reagents. The bases are exceedingly hygroscopic, so that accurate concentrations of solutions for conductance measurement could not be obtained gravimetrically. Solutions made gravimetrically were standardized by titration with $N/50$ hydrochloric acid, using methyl red as indicator.

All conductance measurements were made in a thermostat at $25^\circ \pm .01^\circ \text{C}$. The cell constant was measured in the usual way using pure potassium chloride as electrolyte. Its value for the series of measurements of phenylethyl-isourea was 1.0227. For the measurements of all the other isoureas it was 1.0105. The conductance of the water used in the preparation of solutions was 0.3×10^{-6} mhos.

The data of these experiments are recorded below.

1. *n*-Propyl-isourea

TABLE I

CONDUCTANCE OF *n*-PROPYL-ISOUREA SALICYLATE
(M.P. 146°C .)

Molar concentration	Resistance	Equivalent conductance
0.01	1718	55.81
.005	3291	61.41
.0025	6315	64.00
.0010	15300	67.22
.0000		67.80

$\Lambda_\infty \text{ salt} = 67.80$ $\Lambda_\infty \text{ anion}^* = 34$ $\therefore \Lambda_\infty \text{ cation} = 33.8$
 $\Lambda_\infty \text{ OH}^- = 196$ $\therefore \Lambda_\infty \text{ isourea} = 196 + 33.8 = 229.8$

*Value for salicylate ion: *International Critical Tables*.

TABLE II

CONDUCTANCE OF *n*-PROPYL-ISOUREA

Molar concentration	Resistance	Equivalent conductance	$K \times 10^4$
0.01195	3354	24.64	1.59
.00597	5135	32.93	1.43
.00370	6752	40.44	1.39
.00185	10260	53.23	1.31
Mean			1.43

$K = 1.43 \times 10^{-4}$

2. *n*-Butyl-isourea

TABLE III

CONDUCTANCE OF *n*-BUTYL-ISOUREA SALICYLATE
(M.P. 160°C .)

Molar concentration	Resistance	Equivalent conductance
0.01	1793	56.35
.0025	6520	61.98
.0010	16360	65.78
.0000		66.50

$\Lambda_\infty \text{ salt} = 66.50$ $\Lambda_\infty \text{ anion} = 34$ $\therefore \Lambda_\infty \text{ cation} = 32.50$
 $\Lambda_\infty \text{ OH}^- = 196$ $\therefore \Lambda_\infty \text{ isourea} = 196 + 32.50 = 228.50$

TABLE IV

CONDUCTANCE OF *n*-BUTYL-ISOUREA

Molar concentration	Resistance	Equivalent conductance	$K \times 10^4$
0.04276	1770	13.35	1.55
.02138	2640	17.90	1.43
.01425	3420	20.50	1.30
.01070	4070	23.30	1.31
Mean			1.40

$K = 1.40 \times 10^{-4}$

3. *Isobutyl-isourea*

TABLE V

CONDUCTANCE OF ISOBUTYL-ISOUREA SALICYLATE
(M.P. 164.5° C.)

Molar concentration	Resistance	Equivalent conductance
0.0100	1676	60.28
.0025	6125	66.00
.0010	14600	69.22
.0000	—	70.00

 Λ_{∞} salt 70.00 Λ_{∞} anion 34 $\therefore \Lambda_{\infty}$ cation 36.00
 Λ_{∞} OH⁻ 196 $\therefore \Lambda_{\infty}$ isourea = 196 + 36.00 = 232.00
4. *Isoamyl-isourea*

TABLE VII

CONDUCTANCE OF ISOAMYL-ISOUREA SALICYLATE
(M.P. 155° C.)

Molar concentration	Resistance	Equivalent conductance
0.0100	1593	63.42
.0025	5750	70.30
.0010	13560	74.45
.0000	—	75.20

 Λ_{∞} salt 75.20 Λ_{∞} anion 34.00 $\therefore \Lambda_{\infty}$ cation 41.20
 Λ_{∞} OH⁻ 196 $\therefore \Lambda_{\infty}$ isourea = 196 + 41.20 = 237.20
5. *Allyl-isourea*

TABLE IX

CONDUCTANCE OF ALLYL-ISOUREA SALICYLATE
(M.P. 168° C.)

Molar concentration	Resistance	Equivalent conductance
0.0100	1677	60.25
.0050	3187	63.42
.0025	6100	66.25
.0010	14420	70.10
.0000	—	70.80

 Λ_{∞} salt 70.80 Λ_{∞} anion 34.00 $\therefore \Lambda_{\infty}$ cation 36.80
 Λ_{∞} OH⁻ $\therefore \Lambda_{\infty}$ isourea = 196 + 36.80 = 232.80

TABLE VI

CONDUCTANCE OF ISOBUTYL-ISOUREA

Molar concentration	Resistance	Equivalent conductance	$K \times 10^4$
0.02476	2048	20.43	2.04
.01095	3178	29.20	1.95
.00609	4185	39.60	2.10
.00476	5060	41.93	1.91
Mean			2.00

 $K = 2.00 \times 10^{-4}$

TABLE VIII

CONDUCTANCE OF ISOAMYL-ISOUREA

Molar concentration	Resistance	Equivalent conductance	$K \times 10^4$
0.04020	1946	12.73	1.27
.01886	2837	18.89	1.30
.01190	3582	23.66	1.32
.00457	5917	37.30	1.34
Mean			1.30

 $K = 1.30 \times 10^{-4}$

TABLE X

CONDUCTANCE OF ALLYL-ISOUREA

Molar concentration	Resistance	Equivalent conductance	$K \times 10^4$
0.02823	3520	10.17	5.18
.01410	5182	13.50	5.16
.00941	6590	16.30	4.96
.00706	7700	18.59	4.89
Mean			5.05

 $K = 5.05 \times 10^{-5}$

6. *Phenylethyl-isourea*

TABLE XI

CONDUCTANCE OF PHENYLETHYL-ISOUREA SALICYLATE (M.P. 158° C.)

Molar concentration	Resistance	Equivalent conductance
0.010	1874	54.58
.005	3585	56.98
.001	16300	62.70
.000	—	63.40

Λ_{∞} salt 63.40 Λ_{∞} anion 34.00 $\therefore \Lambda_{\infty}$ cation 29.40
 Λ_{∞} OH⁻ 196 $\therefore \Lambda_{\infty}$ isourea = 196 + 29.40 = 225.40

TABLE XII

CONDUCTANCE OF PHENYLETHYL-ISOUREA

Molar concentration	Resistance	Equivalent conductance	$K \times 10^4$
0.0112	5845	15.90	6.00
.0100	5603	18.02	6.95
.0050	8810	22.93	5.80
.0040	9570	26.40	6.20
Mean			6.23

 $K = 6.23 \times 10^{-4}$
7. *Cyclohexyl-isourea*

TABLE XIII

CONDUCTANCE OF CYCLOHEXYL-ISOUREA HYDROCHLORIDE (M.P. 168° C.)

Molar concentration	Resistance	Equivalent conductance
0.0100	1050	96.22
.0050	2049	98.62
.0025	4010	100.80
.0010	4670	104.45
.0000	—	105.40

Λ_{∞} salt 105.40 Λ_{∞} Cl⁻ 75.90 $\therefore \Lambda_{\infty}$ cation 29.50
 Λ_{∞} OH⁻ 196 $\therefore \Lambda_{\infty}$ isourea = 196 + 29.50 = 225.50

TABLE XIV

CONDUCTANCE OF CYCLOHEXYL-ISOUREA (M.P. 80° C.)

Molar concentration	Resistance	Equivalent conductance	$K \times 10^4$
0.020	2626	19.24	1.59
.010	3810	26.52	1.56
.008	4234	29.83	1.61
.004	6358	39.73	1.54
Mean			1.56

 $K = 1.56 \times 10^{-4}$

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DIFFUSION OF HELIUM THROUGH QUARTZ : RELATION TO TEMPERATURE¹

BY E. F. BURTON,² E. O. BRAATEN,³ AND J. O. WILHELM⁴

Abstract

A qualitative study of the diffusion of certain gases through quartz has been made for ordinary temperatures and pressures.

A nearly linear relation was found to exist between diffusion and pressure for constant temperature and a form of the equation satisfying the experimental results suggested. Temperature-diffusion curves were obtained and a comparison made to previous work. Permeability to helium of single quartz crystalline plates is independent of the direction in which the plate has been cut, but is only about one-half the value of that of fused quartz bulbs or plates.

Introduction

Considerable work has been done on the measurement of the passage of various gases into or through solids at high temperatures; in particular the curious action of quartz towards helium and other gases has received a good deal of attention.

In 1900 Villard (11) found that silica glass was permeable to hydrogen at high temperatures; a little later Jacquerod and Perrot (2) observed that helium leaked out of a thermometer. Since then many workers have obtained both qualitative and quantitative results for the sorption of gases, both by glass and metals. Mayer (6) observed the leakage of hydrogen, nitrogen, and oxygen through quartz at high temperatures and for three different pressures. Richardson (7) and also many others (3, 8, 12, 13, 14) studied the passage of hydrogen and helium through different kinds of glass. In recent work by T'sai and Hogness (10) rates of diffusion, between 180° and 955° C., were obtained for helium passing through quartz and, from 520° to 980° C., for neon, also through quartz. In general it has been found that the rate of diffusion is directly proportional to the pressure and an exponential function of the temperature (4, 9).

Baxter and Starkweather (1) and McLennan and Wilhelm (5) found that diffusion of helium through quartz takes place also at ordinary temperatures and at low temperatures.

Experimental

The work reported in this paper was undertaken in order to investigate carefully and quantitatively the following:—

(1) the diffusion of different gases through quartz at ordinary temperatures; (2) the variation in the diffusion of helium through quartz with pressure, at constant (room) temperature; (3) the variation in the diffusion of helium through quartz with temperature at constant (atmospheric) pressure; (4) the diffusion of helium through crystalline plates of quartz, and a plate of fused quartz at normal pressure and temperature.

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² Contribution from the Physical Laboratories, University of Toronto, Canada.

³ Head of the Department of Physics and Director of the McLennan Laboratory, University of Toronto.

⁴ Holder of a Graduate Fellowship in the Graduate School of the University of Toronto.

⁵ Technical and Research Assistant in the Department of Physics, University of Toronto.

Apparatus and Procedure

Although helium gas passes fairly readily through crystalline quartz, fused quartz and Pyrex glass, it is not able to pass through ordinary soft glass to any noticeable extent. Consequently the general method adopted in these experiments was to maintain a pressure of helium gas inside a quartz bulb which was sealed into an outer chamber of soft glass.

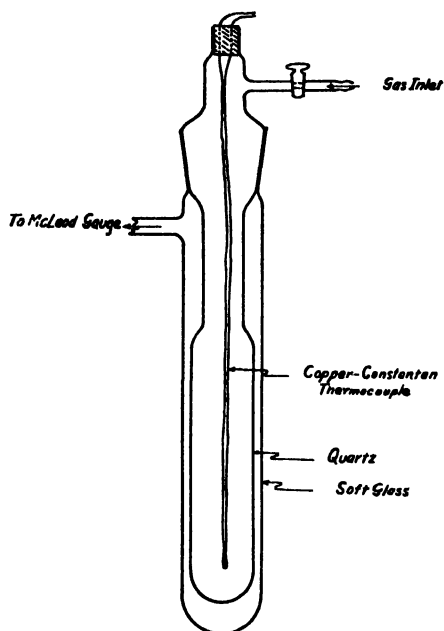


FIG. 1. Apparatus used for quartz bulb.

Fig. 1 shows the arrangement provided for mounting the fused quartz bulb as used in most of the experiments. The quartz container had a total area of 207 sq. cm., the bulb part measuring 3.0 cm. in diameter and 0.80 mm. thick, the neck 1.80 cm. in diameter and 1.36 mm. thick. The quartz tube was fitted with a wax-sealed ground glass joint into a soft-glass container which was connected to a McLeod gauge and diffusion pump. The quartz bulb was kept filled with helium at a given pressure and the soft-glass chamber initially pumped out to a low pressure. Several investigators have shown that the diffusion of helium through soft glass is small, even for high temperatures, so that losses of helium from the low pressure system were negligible.

The thickness of the quartz was obtained by three methods: (a) by the method of volumes, *e.g.*, the difference between the volume displaced on submerging the bulb in a liquid and the volume required to fill the bulb would give the average thickness when divided by the total area; (b) by weight, assuming the density of quartz as 2.2; (c) with a microscope by focusing on the inner and outer surfaces and correcting for the apparent decrease in thickness due to refraction. The three methods checked very well.

Fig. 2 gives the arrangement for a plate of crystalline quartz. Two different plates were obtained from a large single crystal, one cut parallel to, the other perpendicular to, the optic axis. Each was 3.5 cm. in diameter and 1.80 mm. thick. The surfaces were ground with very fine emery and slightly polished with rouge. A small fused quartz plate of the same thickness and 2.5 cm. in diameter, and having received the same surface treatment was also investigated.

Red sealing wax was used for sealing the quartz to the soft glass containers. The pressure was measured on a McLeod gauge which could be read to

0.1 $\times 10^{-4}$ cm. of mercury and a thermostat control served to maintain an oil bath at the required temperature. The temperature of the gas was measured with a thermocouple inside the quartz bulb.

The low pressure system into which the gas diffused was first thoroughly evacuated and tested for leaks. The high pressure side was then pumped out and filled with helium to the required pressure, after first washing this high pressure side of the system two or three times with that gas. Readings were continued at a definite temperature and pressure until a constant rate of increase in pressure was observed, which in some cases took several days.

The rate of diffusion was obtained by calculating the number of cubic centimetres of gas diffusing through per hour per unit surface of wall 1 mm. thick, for a difference in pressure of one atmosphere. Since the temperature was not the same over the whole low pressure system corrections were made. This was easily done by removing the bath and bringing the entire system to room temperature. The two corresponding readings of the pressure gave the correction factor.

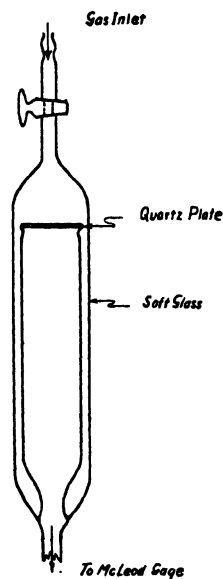


FIG. 2. Apparatus used for quartz plates.

Results

1. The rates of diffusion of air and the two inert gases, neon and argon, were too small to be measured at ordinary temperatures, while the rate for hydrogen was just observable.

TABLE I

RELATION BETWEEN THE DIFFUSION RATE OF HELIUM GAS AND THE PRESSURE AT CONSTANT TEMPERATURE (21° C.)

Pressure, cm. (p)	77.4	59.0	38.6	19.2	5.0
Rate of diffusion $\times 10^6$ (v)	23.4	17.05	10.76	4.98	1.47

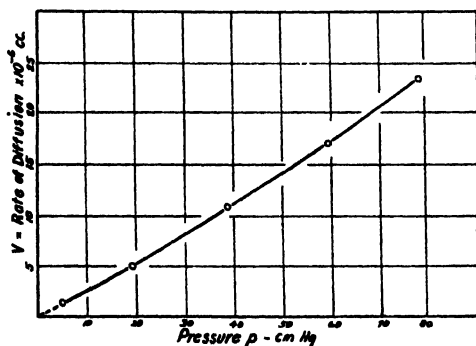


FIG. 3. Curve showing relation between diffusion and pressure at constant temperature.

2. In Table I are recorded the results showing the relation between the diffusion rate of helium gas and the pressure at constant temperature (21° C.); the apparatus is that shown in Fig. 1. In Column 2 the rate of diffusion is calculated in cc. per hour per sq. cm. surface of quartz per mm. thickness.

The curve in Fig. 3 shows graphically the relation between rate of diffusion and pressure. The equation $v = k_1 p + k_2 p^2$ represents the curve, where $k_1 = 0.15$ and $k_2 = 0.0007$.

TABLE II

VARIATION OF RATE OF DIFFUSION OF HELIUM THROUGH QUARTZ WITH CHANGE IN TEMPERATURE FROM $+110^{\circ}$ to -185° C. AT ATMOSPHERIC PRESSURE

Burton, Braaten and Wilhelm			T'sai and Hogness		
Temp., K	Rate of diffusion	Log (rate)	Temp., $^{\circ}$ K	Rate of diffusion	Log (rate)
78	0.87×10^{-8}	-6.0605	453	300.0×10^{-8}	-3.523
195	2.31	-5.7100	583	900	-3.046
232	5.02	-5.2993	713	2,100	-2.678
253.5	7.55	-5.1221	808	3,306	-2.482
273	13.38	-4.8727	858	4,200	-2.377
294	23.0	-4.6383	923	5,100	-2.293
383	121.6	-3.9151	1043	7,200	-2.143
			1153	9,400	-2.027
			1228	11,300	-1.947

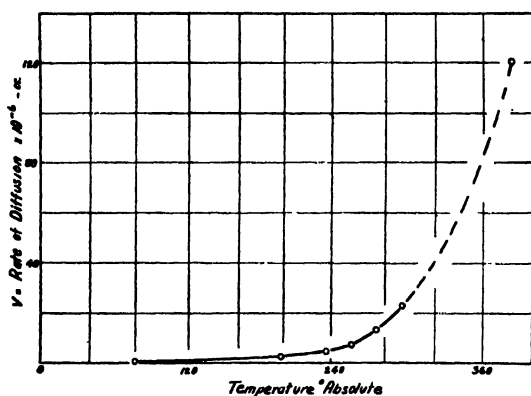


FIG. 4. Curve showing relation between diffusion and temperature at constant pressure.

increasing temperature. In Fig. 5 the temperature was plotted against the logarithm of rate of diffusion. As a means of comparison the results of other investigations were plotted on the same graph with the same scale. After the sudden increase in permeability at -30° C., it then continued to increase rapidly for higher temperatures according to an exponential law up to 80° or 90° C. Williams and Ferguson found an exponential law for high temperatures while an extrapolation of the authors' results fits in well with the work of T'sai and Hogness.

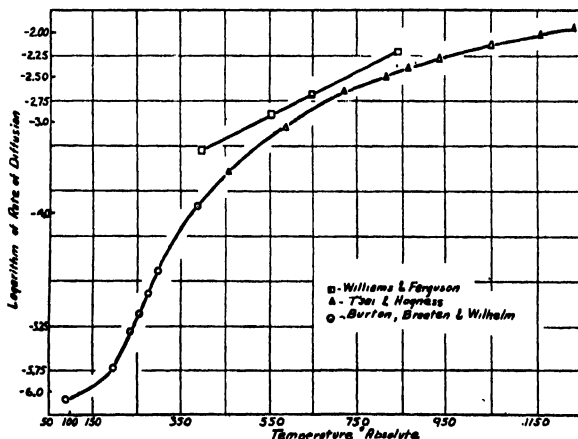


FIG. 5. Same as Fig. 4 showing log (diffusion) and temperature.

4. Diffusion through ground plates of quartz.

In order to find whether there is a difference between fused and crystalline quartz, measurements were made on three ground plates of quartz, as follows (see Fig. 2): (1) A crystalline plate, 3.5 cm. in diameter and 1.85 mm. in thickness, cut perpendicular to the optic axis; (2) A crystalline plate 3.5 cm. in diameter and 1.79 mm. in thickness, cut parallel to the optic axis; (3) A fused quartz plate ground similarly to the others, 2.5 cm. in diameter and 1.75 mm. in thickness.

The resulting rates of diffusion were as follows: (1) 11.8×10^{-6} cc. per hour per sq. cm. per mm. thickness; (2) 12.1×10^{-6} cc. per hour per sq. cm. per mm. thickness; (3) 28.3×10^{-6} cc. per hour per sq. cm. per mm. thickness.

It is interesting to note that the fused quartz ground plate gave only a slightly greater rate than the fused quartz bulb with smooth transparent surfaces; also, that the rates for the crystalline plates are independent of the direction in which the plate is cut and is only about one-half the value for fused quartz.

These results give rise to interesting theoretical speculations which will be reserved for a future paper, when further experiments have been performed.

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A REVIEW OF CANADIAN HELMINTHOLOGY¹

I. THE PRESENT STATUS OF KNOWLEDGE OF THE HELMINTH PARASITES OF DOMESTICATED AND SEMIDOMESTICATED MAMMALS AND ECONOMICALLY IMPORTANT BIRDS IN CANADA, AS DETERMINED FROM WORK PUBLISHED PRIOR TO 1933

By W. E. SWALES²

Abstract

A review is made of all previous records of Trematoda, Cestoda and Nematoda found in Canadian horses, cattle, sheep, swine, deer and buffalo, dogs and cats, foxes, miscellaneous fur-bearers, poultry and game birds.

These records are taken from papers published in various forms which claim identification of the parasites found, and are here published as check lists following an introduction and a semihistorical review of Canadian helminthology.

The purpose of this paper is to compile all the data published concerning definite records of helminths parasitic in important animals and birds in Canada, with a view to ascertaining their distribution and known importance at the time this Institute commenced to function actively. At the same time, the results of the author's observations concerning the prevalence of these parasites will be recorded as a second part of the paper, and thus the two parts together will summarize the extent of our knowledge of this subject at the end of the year 1932. Thereafter, all work published will be considered as building upon this foundation and, it is hoped, will lead to a thorough knowledge of the prevalence and importance of each species of disease-causing helminths in Canada.

It has been said that time spent on survey work is time wasted for the reason that all really important parasites show themselves to be present as soon as disease symptoms are observed. The exponents of this view have apparently not followed the results of recent investigations in helminthology because it has been conclusively shown that many parasites play roles in the etiology of disease that are commonly overlooked; furthermore, a knowledge of their distribution is absolutely necessary if we are to know their life cycles under different conditions and in widely separated regions.

Agricultural science has shown how large numbers of animals and birds may be kept on small areas and thus parasitic diseases have increased tremendously because the hazards usually encountered by a worm during its life cycle have been materially decreased. Wild animals have been taken from their natural environs and confined in small pens for the domestic production of furs and have become the prey of numerous diseases caused by excessive numbers of helminths which, under natural conditions, would live

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in the host without causing any untoward effect. Hunters have driven huge flocks of ducks and geese into every small area where food can be obtained in safety, such as private game reserves and national parks. There, the more or less normal inhabitants of the birds' intestinal tracts continue to lay millions of eggs which would normally be scattered over huge areas, but which under modern conditions are spread over these comparatively small areas. Thus abnormally large infestations occur which cause serious disease symptoms, often so serious as to eventually bring about the death of the host.

In making such a survey, it is impossible to limit it to species known to cause disease or to live in economically important animals. Many of the species in domestic animals, for example, live also in wild herbivores and carnivores. The larval stages of tapeworms of wild dogs and cats, for example, may develop as huge parasitic cysts in domestic animals. Modern transport facilitates the transference of both host and parasite from one part of the country to another. New hosts, newly introduced into the Dominion, may contract parasites already existing in our native animals, and parasitic disease in a new species of host is always much more severe than in an old, normal one. Alterations in food supply may lead an animal to contract a strange parasite, with similar results. These reasons for widening and extending the scope of a survey to include all economic animals and their wild relations, could be considerably multiplied.

Of even more importance is the fact that helminthology, like all other branches of science, cannot be understood unless all branches are studied. This has been proved by many instances in the past where important clues to the unravelling of life cycles of parasites of man and valuable animals have been obtained through the study of the parasites of creatures which are of less economic importance. For instance, Cameron's discovery that the lungworm of the cat required an intermediate host led to the discovery that many other lungworms had similar life cycles, and thus effective control measures can now be employed.

Leuckart's work in Germany on the larva of *Cucullanus elegans*, a parasite of the perch, provided the clue that resulted in the life history of the Guinea worm being worked out in Turkestan by Fedtschenko in 1869. The discovery of the life cycle of *Fasciola* was the result of purely academic studies on pond life which enabled Thomas and Leuckart to obtain the knowledge concerning this complicated cycle, and which is now of immense economic value.

Looss' work on the hookworm of man, *A. duodenale*, was largely controlled and supported by his parallel experiments on *A. caninum* in dogs.

The writer hopes that this urgent need for survey work will be realized by workers in the fields of agricultural, veterinary, medical and other biological sciences in Canada, and that all will co-operate by placing on definite record, the parasites of Canadian mammals and birds, either through the medium of independent publications or by submitting material to a qualified helminthologist, with descriptions of location, locality and apparent effect upon the host.

Canadian Parasitologists

The observations of Sir William Osler on the identity and prevalence of certain helminth parasites of man and animals in Canada comprise the first records to be made in the Dominion, and it is with this work we begin our search for data concerning Canadian helminthology. Besides recording many parasites of domesticated animals, Osler published notes and papers dealing with helminths of man, chief among which are *Trichinella spiralis* (Toronto, 1870), *Enterobius vermicularis*, *Taenia saginata*, *Taenia solium* and *Filaria bancrofti* (Montreal, 1885-1890).

Many years elapsed before any further publications on the subject appeared in Canada; in fact, barring Stafford of McGill and Wright of Toronto, who wrote several notes on helminths of reptiles and batrachians in the early part of this century, the next worker of note was Dr. Seymour Hadwen, present Director of the Veterinary Department, Ontario Research Foundation. Although Hadwen's chief work was in the field of medical and veterinary entomology, he has added several valuable records to Canadian helminthology; and the paper by Ransom and Hadwen on Horse Strongyles in Canada, which appeared in 1920, gives us an excellent record of this group in Saskatchewan and Alberta.

In 1920 and 1922 the importance of the parasites of poultry and domesticated foxes was realized by several workers, and mention was made of this group many times. The most reliable records from a distribution point of view were those furnished by J. A. Allen, A. B. Wickware and G. Ennis Smith, all of whom made valuable additions to our collection of data.

Several years after this period other workers appeared in the field, and although little original work was published, it was evident that helminthology was becoming recognized as a very important science in Canada. Baker and Conklin, working at Macdonald College, published several notes on the occurrence and prevalence of parasites in domestic birds and in one instance (1930) of sheep, while Kingscote, Schofield, Batt and McIntosh all made contributions through the medium of the Animal Report of the Ontario Veterinary College.

Ronald Law, Director of the Experimental Fur Farm of the Ontario Department of Game and Fisheries, has taken an active interest in parasites of fur-bearing animals for the last three or four years. As well as doing extensive experimental control work he, and his coworkers, have made very complete records of the helminths of this group of animals in Ontario, and published in 1932 a descriptive bulletin with check lists of the parasites of the various fur-bearing hosts.

Several scientists in the United States have from time to time given their attention to parasites from Canadian sources and in some instances have described them as new species. These workers include Ransom, Hall, Price, Cram and Chapin, and also several others who paid attention to helminths not included under the heading of this paper.

It will be noted that the popular publications in which many important helminths are mentioned have not been included in this paper. It is obvious

that in this list can be included only parasites that have been definitely identified and recorded from a province, and these papers make no claim to definite identification but merely quote the probable facts that the parasites are exceedingly prevalent.

Check List of Helminth Parasites of Economically Important Mammals and Birds in Canada

This list is comprised of only such parasites as have been recorded from time to time as definitely identified species by many workers in Canada and elsewhere during the last fifty years.

For the sake of convenience, the parasites are listed under the headings of hosts, and merely subdivided under the Classes to which they belong.

HORSES (*Equus caballus*)

Parasite	First recorded by	Province	Year	Parasite	First recorded by	Province	Year
CESTODA							
<i>Anoplocephala mamillana</i>	Kingscote Bruce	Ont. B.C.	1931 1930	<i>Anoplocephala magna</i>	Kingscote	Ont.	1931
NEMATODA							
<i>Ascaris equorum</i>	Ransom & Hadwen Bruce	Sask. and Alta. B.C.	1917 1930	<i>Cylicostomum nassatum</i>	Ransom & Hadwen	Sask. and Alta.	1917
<i>Oxyuris equi</i>	Bruce	B.C.	1930	<i>Cylicostomum elongatum</i>	Ransom & Hadwen	Sask.	1917
<i>Strongylus equinus</i>	Ransom & Hadwen	Sask. and Alta.	1917	<i>Cylicostomum insigne</i>	Ransom & Hadwen	Alta.	1917
<i>Strongylus edentatus</i>	Ransom & Hadwen Schofield	Sask. and Alta. Ont.	1917 1928	<i>Cylicostomum goldi</i>	Ransom & Hadwen	Sask. and Alta.	1917
<i>Strongylus vulgaris</i>	Ransom & Hadwen Schofield Bruce	Sask. and Alta. Ont. B.C.	1917 1928 1930	<i>Cylicostomum leptostomum</i>	Cram	Sask.	1923
<i>Cylicostomum labiatum</i>	Ransom & Hadwen	Sask. and Alta.	1917	<i>Cylicostomum ultrajectinum</i>	Cram	Sask.	1923
<i>Cylicostomum labratum</i>	Ransom & Hadwen	Sask. and Alta.	1917	<i>Cylicostomum spp.</i>	Schofield	Ont.	1928
<i>Cylicostomum poculatum</i>	Ransom & Hadwen	Sask. and Alta.	1917	<i>Oesophagodontus robustus</i>	Ransom & Hadwen	Sask. and Alta.	1917
<i>Cylicostomum catinatum</i>	Ransom & Hadwen	Sask. and Alta.	1917	<i>Gyalocephalus capitatus</i>	Ransom & Hadwen	Sask. and Alta.	1917
<i>Cylicostomum coronatum</i>	Ransom & Hadwen	Sask.	1917	<i>Triodontophorus brevicauda</i>	Ransom & Hadwen	Sask. and Alta.	1917
<i>Cylicostomum bicoronatum</i>	Ransom & Hadwen	Sask. and Alta.	1917	<i>Triodontophorus intermedius</i>	Ransom & Hadwen	Sask. and Alta.	1917
<i>Cylicostomum calicatum</i>	Ransom & Hadwen	Sask. and Alta.	1917	<i>Triodontophorus tenuicollis</i>	Ransom & Hadwen	Sask. and Alta.	1917
<i>Cylicostomum radiatum</i>	Ransom & Hadwen	Sask. and Alta.	1917				

NOTE:—In the above records the members of the sub-family Trichoneminae are listed under the old generic name *Cylicostomum*. Owing to differences of opinion concerning the nomenclature of this group it was considered that this plan would suffice.

CATTLE (*Bos taurus*)

Parasite	First recorded by	Province	Year	Parasite	First recorded by	Province	Year
TREMATODA							
<i>Paramphistomum cervi</i>	Osler Bruce	N.S. B.C.	1882 1930	<i>Fascioloides magna</i>	Bruce	B.C.	1930
				<i>Fasciola hepatica</i>	Hadwen	B.C.	1916
CESTODA							
<i>Moniezia expansa</i>	Bruce	B.C.	1930	<i>Echinococcus granulosus</i> (cyst.)	Osler Bruce	Que. B.C.	1880 1930
<i>Cysticercus bovis</i>	Osler Bruce	Que. B.C.	1883 1930				
NEMATODA							
<i>Dictyocaulus viviparus</i>	Bruce	B.C.	1930	<i>Oesophagostomum radiatum</i>	Bruce	B.C.	1930
<i>Gongylonema scutatum</i>	Bruce	B.C.	1930				

SHEEP (*Ovis aries*)

Parasite	First recorded by	Province	Year	Parasite	First recorded by	Province	Year
TREMATODA							
<i>Fasciola hepatica</i>	Hadwen	B.C.	1916	<i>Dicrocoelium dendriticum</i>	Conklin & Baker	Que.	1930
CESTODA							
<i>Moniezia expansa</i>	McIntosh Bruce	Ont. B.C.	1927 1930	<i>Cysticercus tenuicollis</i>	Bruce	B.C.	1930
<i>Thysanosoma actinoides</i>	Bruce	B.C.	1930	<i>Cysticercus ovis</i>	Bruce	B.C.	1930
NEMATODA							
<i>Trichuris ovis</i>	Bruce	B.C.	1930	<i>Monodontus trigonocephalus</i>	Bruce	B.C.	1930
<i>Dictyocaulus filaria</i>	McIntosh Bruce	Ont. B.C.	1928 1930	<i>Oesophagostomum columbianum</i>	Bruce	Eastern Canada	1930
<i>Protostrongylus rufescens</i> (?)	Bruce	B.C.	1930				
<i>Nematodirus</i> sp.	Bruce	B.C.	1930	<i>Haemonchus contortus</i>	Bruce Schofield	B.C. Ont.	1930 1924

SWINE (*Sus scrofa domesticus*)

Parasite	First recorded by	Province	Year	Parasite	First recorded by	Province	Year
CESTODA							
<i>Cysticercus cellulosae</i>	Bruce	B.C.	1930	<i>Echinococcus granulosus</i> (cyst.)	Osler Bruce	Que. B.C.	1880 1930
NEMATODA							
<i>Ascaris lumbricoides</i>	McIntosh Bruce	Ont. B.C.	1928 1930	<i>Metastrongylus elongatus</i>	Bruce	B.C.	1930
<i>Dochmoides stenocephala</i>	Ransom	Ont.	(?)	<i>Trichuris suis</i>	Bruce	B.C.	1930
				<i>Trichinella spiralis</i>	Osler	Que.	1880

DEER AND BUFFALO				
Parasite	Host	First recorded by	Province	Year
TREMATODA				
<i>Fascioloides magna</i>	Odocoileus columbianus	Hadwen	B.C.	1916
	Bison bison	Cameron	Alta.	1923
<i>Fasciola hepatica</i>	Odocoileus columbianus	Hadwen	B.C.	1916
CESTODA				
<i>Moniezia</i> sp.	Bison bison	Cameron	Alta.	1923
<i>Echinococcus granulosus</i>	Alces alces	Hadwen	Man.	1932
<i>Cysticercus tenuicollis</i>	Cervus elaphus	Hadwen	Alta.	1932
NEMATODA				
<i>Cooperia bisonis</i>	Bison bison	Cram	Alta.	1925
<i>Ostertagia bisonis</i>	Bison bison	Chapin	Alta.	1925
<i>Dictyocaulus hadweni</i>	Bison bison	Chapin	Alta.	1925
<i>Dictyocaulus filaria</i>	Deer	Bruce	B.C.	1930
<i>Filaria</i> sp.	Deer	Bruce	B.C.	1930

DOGS AND CATS (*Canis familiaris* and *Felis catus domesticus*)

Parasite	Host	First recorded by	Province	Year
CESTODA				
<i>Dipylidium caninum</i>	Dog	Osler	Que.	1877
	Dog	Bruce	B.C.	1930
<i>Taenia taeniaformis</i>	Cat	Kingscote	Ont.	1931
<i>Taenia pisiformis</i>	Dog	McIntosh	Ont.	1927
	Dog	Bruce	B.C.	1930
<i>Taenia serialis</i>	Dog	Bruce	B.C.	1930
<i>Taenia hydatigena</i>	Dog	Bruce	B.C.	1930
<i>Echinococcus granulosus</i> (?)	Dog	Bruce	B.C.	1930
<i>Diphyllobothrium latum</i>	Dog	Vergeer	Alta., Man., Ont.	1928
NEMATODA				
<i>Toxascaris limbata</i>	Dog	Bruce	B.C.	1930
	Dog	Osler	Que.	1877
<i>Belascaris marginata</i>	Dog	Bruce	B.C.	1930
<i>Ancylostoma caninum</i>	Dog	Osler	Que.	1877
<i>Trichuris vulpis</i>	Dog	Osler	Que.	1877
<i>Oslerus osleri</i>	Dog	Osler	Que.	1877
<i>Diocotphyne renale</i>	Dog	Riley	(?)	1924

FOXES (*Vulpes fulva*)

Parasite	First recorded by	Province	Year	Parasite	First recorded by	Province	Year
TREMATODA							
<i>Parascacotyle longa</i>	Allen & Wickware	—	1922	<i>Alaria americana</i>	Law & Kennedy	Ont.	1932
<i>Echinochasmus</i> sp.	Allen & Wickware	—	1922	<i>Alaria arisaemoides</i>	Law & Kennedy	Ont.	1932
				<i>Paragonimus kellicotti</i>	Kingscote	Ont.	1931

FOXES (*Vulpes fulva*)—Concluded

Parasite	First recorded by	Province	Year	Parasite	First recorded by	Province	Year
CESTODA							
<i>Diphyllobothrium latum</i>	Law & Kennedy	Ont.	1932	<i>Diphyllobothrium cordatum</i>	Law & Kennedy	Ont.	1932
NEMATODA							
<i>Belascaris marginata</i>	Allen & Wickware	—	1922	<i>Dochmoides stenocephala</i>	Allen & Wickware	—	1922
	Law & Kennedy	Ont.	1932		Law Bruce	Ont. B.C.	1930 1930
	Bruce	B.C.	1930	<i>Ancylostomum caninum</i>	Batt Bruce	Ont. B.C.	1925 1930
<i>Belascaris mystax(?)</i>	Higgins	Ont.	1916	<i>Crenosoma decoratum</i>	Law	Ont.	1931
	Allen & Wickware	—	1922	<i>Capillaria plica</i>	Law	Ont.	1931
<i>Toxascaris limbata</i>	Allen & Wickware	—	1922	<i>Eucoleus aerophilus</i>	Batt Bruce	Ont. B.C.	1924 1930
	Law	Ont.	1931				

MISCELLANEOUS FUR-BEARING ANIMALS

The following parasites of fur-bearers were recorded by Law and Kennedy, 1932, as helminths infesting fur-bearing animals in the vicinity of Kirkfield, Ontario.

Parasite	Hosts	Parasite	Hosts
TREMATODA			
<i>Plagiorchis proximus</i>	Mink (<i>Mustela vison</i>) Muskrat (<i>Ondatra zibethica</i>)	<i>Echinostomum callawayensis</i>	Muskrat (<i>Ondatra zibethica</i>)
<i>Parametorchis canadensis</i>	Mink (<i>Mustela vison</i>)	<i>Echinoparyphium contiguum</i>	Muskrat (<i>Ondatra zibethica</i>)
<i>Notocotyle quinqueseptale</i>	Muskrat (<i>Ondatra zibethica</i>)	<i>Echinostomum armigerum</i>	Muskrat (<i>Ondatra zibethica</i>)
<i>Nudacotyle novicia</i>	Muskrat (<i>Ondatra zibethica</i>)	<i>Psilostomum ondatrae</i>	Muskrat (<i>Ondatra zibethica</i>)
<i>Cotatropis filamentis</i>	Muskrat (<i>Ondatra zibethica</i>)	<i>Hemistomum craterum</i>	Muskrat (<i>Ondatra zibethica</i>)
<i>Echinochasmus schwartzi</i>	Muskrat (<i>Ondatra zibethica</i>)	<i>Alaria mustelae</i>	Muskrat (<i>Ondatra zibethica</i>)
<i>Euparyphium melis</i>	Mink (<i>Mustela vison</i>)	<i>Alaria mustelae</i>	Mink (<i>Mustela vison</i>)
<i>Euparyphium inerme</i>	Mink (<i>Mustela vison</i>)	<i>Alaria americana</i>	Wolf (<i>Canis lycaon</i>)
<i>Echinostomum coalitum</i>	Muskrat (<i>Ondatra zibethica</i>)	<i>Wardius zibethicus</i>	Muskrat (<i>Ondatra zibethica</i>)
		<i>Cladorchis subtriquetrus</i>	Beaver (<i>Castor canadensis</i>)
CESTODA			
<i>Hymenolepis evaginata</i>	Muskrat (<i>Ondatra zibethica</i>)	<i>Taenia</i> sp.	Mink (<i>Mustela vison</i>)
<i>Taenia pisiformis</i>	Wolf (<i>Canis lycaon</i>)	<i>Cysticercus fasciolaris</i>	Muskrat (<i>Ondatra zibethica</i>)

MISCELLANEOUS FUR-BEARING ANIMALS—*Concluded*

Parasite	Hosts	Parasite	Hosts
NEMATODA			
<i>Toxocara mystax</i>	Lynx (<i>Lynx canadensis</i>)	Physaloptera sp.	Lynx (<i>Lynx canadensis</i>)
<i>Ascaris</i> sp.	Mink (<i>Mustela vison</i>)	Physaloptera sp.	Raccoon (<i>P. lotor</i>)
<i>Ascaris</i> sp.	Raccoon (<i>P. lotor</i>)	Capillaria sp.	Raccoon (<i>P. lotor</i>)
<i>Strongyloides</i> sp.	Mink (<i>Mustela vison</i>)	Capillaria sp.	Mink (<i>Mustela vison</i>)
<i>Filaria</i> sp.	Mink (<i>Mustela vison</i>)	<i>Diectophyme renale</i>	Mink (<i>Mustela vison</i>)
<i>Filarioides bronchialis</i>	Mink (<i>Mustela vison</i>)	<i>Hepaticola hepaticola</i>	Muskrat (<i>Ondatra zibethica</i>)

It must be noted the trematodes, *Parametorchis canadensis* and *Psilostomum ondatrae*, were new species described by Price, and therefore first reported by him in 1929 and 1931 respectively. Added to the above list is *Paragonimus kellicotti* which was first recorded in an Ontario mink in 1931 by Kingscote: also *Dochmoides stenocephala* in a Fisher (*Mustela* sp.) by Chitwood, 1932.

BIRDS (*Domestic and Game*)

Parasite	Host	First recorded by	Province	Year
CESTODA				
<i>Davainea proglottina</i>	<i>G. gallus</i>	Wickware	—	1922
	<i>G. gallus</i>	Gwatkin	Ont.	1924
	<i>G. gallus</i>	Baker	Que.	1930
	<i>G. gallus</i>	Bruce	B.C.	1930
<i>Raillietina tetragona</i>	<i>G. gallus</i>	Wickware	—	1922
<i>Amoebotaenia sphenoides</i>	<i>G. gallus</i>	Wickware	—	1922
	<i>G. gallus</i>	Baker	Que.	1930
<i>Raillietina cesticillus</i>	<i>G. gallus</i>	Wickware	—	1922
	<i>G. gallus</i>	Baker	Que.	1930
<i>Choantaenia infundibulum</i>	<i>Anas dom.</i>	Wickware	—	1922
	<i>Gallus</i> sp.	Bruce	B.C.	1930
	<i>G. gallus</i>	Baker	Que.	1930
<i>Hymenolepis carioca</i>	<i>G. gallus</i>	Wickware	—	1922
	<i>G. gallus</i>	Baker	Que.	1930
<i>Hymenolepis coronula</i>	<i>Anas dom.</i>	Schofield	Ont.	1931
NEMATODA				
<i>Ascaridia lineata</i>	<i>G. gallus</i>	Wickware	—	1922
	<i>G. gallus</i>	Baker	Que.	1930
	<i>G. gallus</i>	Bruce	B.C.	1930
<i>Ascaridia columbae</i>	<i>Columba livia</i>	Wickware	—	1922
	<i>Columba livia</i>	Rayner	Que.	1932
<i>Heterakis gallinae</i>	<i>G. gallus</i> , <i>Meleagris dom.</i>	Wickware	—	1922
	<i>G. gallus</i>	Baker	Que.	1930
	<i>G. gallus</i>	Gwatkin	Ont.	1924
	<i>G. gallus</i>	Bruce	B.C.	1930
<i>Heterakis isolonche</i>	<i>Phasianus</i> sp.	Schofield	Ont.	1924

BIRDS (*Domestic and Game*)—Concluded

Parasite	Host	First recorded by	Province	Year
NEMATODA				
<i>Syngamus trachealis</i>	Gallus sp.	Wickware	—	1922
<i>Capillaria retusa</i>	<i>G. gallus</i>	Wickware	—	1922
<i>Capillaria annulata</i>	<i>G. gallus</i>	Wickware	—	1922
<i>Capillaria meleagrisgallopava</i>	<i>G. gallus</i>	Baker	Que.	1930
<i>Capillaria columbae</i>	<i>Columba livia</i>	Rayner	Que.	1932
<i>Capillaria</i> sp.	<i>G. gallus</i>	Gwatkin	Ont.	1924
		Bruce	B.C.	1930
<i>Chielospirura hamulosa</i>	<i>G. gallus</i>	Schofield	Ont.	1930
		Conklin	Que.	1932
<i>Amidostomum anseris</i>	Branta sp.	Cram	Ont.	1928
<i>Echinuria parva</i>	Branta sp.	Cram	Ont.	1928

A list of parasites collected in Canada and present in the collection of the Zoological Division, United States Bureau of Animal Industry, Washington, D.C., on May 1, 1931, just before the author commenced his first observations upon the distribution of Canadian helminths, and is here reproduced by the kind permission of Dr. Maurice C. Hall. This list is limited to the helminth parasites of domesticated animals and birds, economically important fur-bearers and game birds and mammals.

Parasite	Host	Province of origin	Collection number
TREMATODA			
<i>Plagiorchis proximus</i>	Muskrat (<i>O. zibethica</i>)	Ont.	464-13
<i>Dicrocoelium dendriticum</i>	Sheep	Que.	2521-A
<i>Parametorchis intermedius</i>	Mink (<i>Mustela vison</i>)	Que.	2521-B
<i>P. canadensis</i>	Mink (<i>Mustela vison</i>)	Ont.	464-B
<i>Cryptocotyle lingua</i>	Fox (<i>V. fulva</i>)	P.E.I.	468-1
<i>Nudacotyle novicia</i>	Muskrat (<i>O. zibethica</i>)	Ont.	2550-D
<i>Fascioloides magna</i>	Deer	B.C.	1748-E
<i>Echinostomum revolutum</i>	Muskrat (<i>O. zibethica</i>)	Ont.	2199-D
<i>E. echinatum</i>	Goose (<i>Anser cinerius</i>)	Ont.	2135-A
<i>Echinoparyphium contiguum</i>	Muskrat (<i>Mustela vison</i>)	Ont.	464-11
<i>Psilostomum ondatrae</i>	Muskrat (<i>O. zibethica</i>)	Ont.	T. 133-1
<i>Alaria americana</i>	Fox (<i>V. fulva</i>)	Ont.	2216-C
<i>Alaria alata</i>	Fox (<i>V. fulva</i>)	Ont.	453-11
<i>Cladorchis subtriquetrus</i>	Beaver (<i>C. canadensis</i>)	Ont.	2648-A
CESTODA			
<i>Anoplocephala</i> sp.	Horse	Ont.	2154-B
<i>Moniezia expansa</i>	Sheep	—	L. 1270-B
<i>Moniezia</i> sp.	Bison	Alta.	55-1E
<i>Thysanosoma actinoides</i>	Sheep	B.C.	1727-C
<i>Davainea proglottina</i>	Chicken (dom.)	Que.	
<i>Railletina cesticillus</i>	Chicken (dom.)	Que.	2135-B
<i>Hymenolepis evaginata</i>	Muskrat (<i>O. zibethica</i>)	Ont.	2470-E
<i>Taenia pisiformis</i>	Fox (<i>V. fulva</i>)	Ont.	2452-B

Parasite	Host	Province of origin	Collection number
NEMATODA			
<i>Ascaris lumbricoides</i>	Pig	Ont.	2452-D
<i>Ascaris</i> sp.	Raccoon (<i>P. lotor</i>)	Ont.	2527-E
<i>Ascaridia lineata</i>	Fowl (<i>G. gallus</i>)	Que.	
<i>Heterakis gallinae</i>	Fowl (<i>G. gallus</i>)	Que.	
<i>Heterakis isolonche</i>	Pheasant sp.	Ont.	1696-B
<i>Probstmayria vivipara</i>	Horse	—	2328-B
<i>Strongylus equinus</i>	Horse	Sask.	2173
<i>Strongylus vulgaris</i>	Horse	Sask.	2173
<i>Strongylus edentatus</i>	Horse	Sask.	2173
<i>Triodontophorus intermedius</i>	Horse	Sask.	2174
<i>Warrenius bifurcatus</i> (?)	Horse	Sask.	Cotype
<i>Citellus richardsoni</i>	Horse	Sask.	T. 15-A
<i>Oesophagostomum radiatum</i>	Bison	Alta.	2137-B
NEMATODA			
Cylicostomum spp. (As reported, Ransom and Hadwen 1918).			
<i>Dictyocaulus hadweni</i>	Bison	Alta.	T. 10-A
<i>Trichostrongylus colubriiformis</i>	Sheep	Ont.	2452-C
<i>T. vitrinus</i>	Sheep	Ont.	2452-C
<i>Cooperia bisonis</i>	Bison	Alta.	T. 121-10
<i>Ostertagia bisonis</i>	Bison	Alta.	T. 10-D
<i>Crenosoma</i> sp.	Fox (<i>V. fulva</i>)	Ont.	2657-E
<i>Amidostomum anseris</i>	Goose (<i>Branta</i> sp.)	Ont.	2231-D
<i>Filaria</i> sp.	Deer (<i>O. columbianus</i>)	B.C.	2297-C
<i>Setaria labiato-papillosa</i>	Bison	Alta.	1051-E
<i>Dispharynx spiralis</i>	Grouse (<i>Bonasa umbellus</i>)	N.S.	2659-D
<i>Echinuria parva</i>	Goose (<i>Branta</i> sp.)	Ont.	T. 127-5
<i>Physaloptera</i> sp.	Raccoon (<i>P. lotor</i>)	Ont.	2657-D
<i>Diectophyme renale</i>	Mink (<i>M. vison</i>)	Ont.	2639-B
<i>Capillaria plica</i>	Fox (<i>V. fulva</i>)	Ont. and P.E.I.	2042-D
<i>Capillaria</i> sp.	Mink (<i>M. vison</i>)	Ont.	—
<i>Capillaria meagris-gallo-pavo</i>	Fowl (<i>G. gallus</i>)	Que.	—
<i>Eucoleus aerophilus</i>	Fox (<i>V. fulva</i>)	(?)	1951-B

NOTE:—The bibliography for this paper is included in Part II.

A REVIEW OF CANADIAN HELMINTHOLOGY

II. ADDITIONS TO PART I, AS DETERMINED FROM A STUDY OF PARASITIC HELMINTHS COLLECTED IN CANADA¹

By W. E. SWALES²

Abstract

The author adds to Part I further records of Trematoda, Cestoda and Nematoda of Canadian horses, cattle, swine, sheep, poultry, game birds and miscellaneous hosts. These appear as check lists, the specimens having been identified by the author and deposited in helminthological collections. Parts I and II summarize the extent of knowledge concerning the distribution and prevalence of these parasites in Canada, and a bibliography covering these papers is herewith included.

The following check lists of parasitic helminths were compiled following taxonomic studies made by the author upon specimens collected in Canada. Many of these parasites were taken from the collection of the Animal Diseases Research Institute, Hull, Que., which was made available to this Institute through the co-operation of Dr. E. A. Watson, Health of Animals Branch, Department of Agriculture. Others were collected by the author or submitted for identification.

The helminths previously listed in Part I, which took the form of a review of Canadian helminthology, have not been again noted in this paper except as records for other provinces. The specimens listed below have been systematically studied and deposited in the helminthological collections of the aforementioned Institutes.

The two parts of this preliminary survey work summarize the extent of our knowledge concerning the distribution and prevalence of these parasites in Canada at the end of the year 1932, and will show how urgently we need to gain further knowledge of this subject.

HORSE (<i>Equus caballus</i>)					
Parasite	Provinces	Apparent prevalence	Parasite	Provinces	Apparent prevalence
CESTODA					
<i>Anoplocephala mamillana</i>	B.C., Sask., Que.	Fairly common	<i>Anoplocephala perfoliata</i>	Ont.	One specimen examined
<i>Anoplocephala magna</i>	Que.	(?)			
NEMATODA					
<i>Ascaris equorum</i>	Ont., Que.	Common	<i>Triodontophorus brevicauda</i>	Que.	(?)
<i>Probstmayria vivipara</i>	Que. (Hull)	(?)	<i>Gyaloccephalus capitatus</i>	Ont.	(?)
<i>Oxyuris equi</i>	Ont., Que.	Common	<i>Oesophagodontus robustus</i>	Ont., Que.	(?)
<i>Strongylus equinus</i>	Ont., Que.	Common	<i>Dictyocaulus arnfieldi</i>	Ont.	Not common
<i>Strongylus vulgaris</i>	Ont., Que.	Common			
<i>Strongylus edentatus</i>	Que.	(?)			

¹ Manuscript received March 9, 1933.

Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada and the Empire Marketing Board.

² Research Assistant.

HORSE (*Equus caballus*)—Concluded

Parasite	Provinces	Apparent prevalence	Parasite	Provinces	Apparent prevalence
NEMATODA					
<i>Trichonema</i> spp. (10)	Ont., Que.	Estimation not attempted (?)	<i>Setaria equina</i>	Ont., Que.	Fairly common
<i>Triodontophorus serratus</i>	Ont., Que.		<i>Habronema muscae</i>	Ont.	Not common
			<i>Habronema microstoma</i>	Ont.	(?)

CATTLE (*Bos taurus*)

Parasite	Provinces	Apparent prevalence	Parasite	Provinces	Apparent prevalence
TREMATODA					
<i>Paramphistomum</i> sp.	Ont.	Rare	<i>Fascioloides magna</i>	Alta.	Common
<i>Paramphistomum</i> sp.	Alta.	(?)			
CESTODA					
<i>Moniezia benedeni</i>	Que.	(?)	<i>Cysticercus tenuicollis</i>	Ont., Que.	Common
<i>Moniezia expansa</i>	Que.	Common	<i>Cysticercus bovis</i>	Ont., Sask.	Fairly common
NEMATODA					
<i>Oesophagostomum radiatum</i>	Ont., Que.	Common	<i>Cooperia punctata</i>	Ont. Que.	Uncommon
<i>Monodontus phlebotomus</i>	Que.	(?)	<i>Ostertagia ostertagi</i>		Uncommon
<i>Monodontus trigonocephalus</i>	Ont.	(?)	<i>Haemonchus contortus</i>	Ont., Que.	Common
<i>Dictyocaulus viviparus</i>	Ont., Que.	(?)	<i>Setaria labiato-papillosa</i>	Ont., Que., Alta., Man.	Common
			<i>Trichuris ovis</i>	Que.	(?)

SHEEP (*Ovis aries*)

Parasite	Provinces	Apparent prevalence	Parasite	Provinces	Apparent prevalence
CESTODA					
<i>Moniezia expansa</i>	Ont., Que.	Common	<i>Cysticercus tenuicollis</i>	Ont., Que., Alta.	Very common
<i>Thysanosoma actinioides</i>	Sask., Alta.	Very common in Mid-West	<i>Cysticercus ovis</i>	Man.	(?)
NEMATODA					
<i>Oesophagostomum columbianum</i>	Ont., Que., N.S.	Causing severe losses in Eastern Canada	<i>Nematodirus spathiger</i>	Ont.	Fairly common
<i>Oesophagostomum venulosum</i>	Ont., Que.	Two cases seen	<i>Nematodirus filicollis</i>	Ont.	(?)
<i>Chabertia ovina</i>	Ont.	One case	<i>Ostertagia circumcincta</i>	Ont.	(?)
<i>Monodontus trigonocephalus</i>	Ont., Que.	Very common	<i>Haemonchus contortus</i>	Que.	A dominion-wide menace
<i>Dictyocaulus filaria</i>	Ont., Que., P.E.I.	(?)	<i>Haemonchus</i> sp.	Ont.	Only females available
<i>Trichostrongylus extenuatus</i>	Ont.	(?)	<i>Gongylonema pulchrum</i>	Ont.	Rare
<i>Cooperia curticei</i>	Ont.	(?)	<i>Trichuris ovis</i>	Ont., Que., N.S.	Common
<i>Cooperia oncophora</i>	Ont.	(?)			

SWINE (*Sus scrofa domesticus*)

Parasite	Provinces	Apparent prevalence	Parasite	Provinces	Apparent prevalence
CESTODA					
<i>C. cellulosae</i>	Man., Sask.	Now rare			
NEMATODA					
<i>Ascaris lumbricoides</i>	All provinces	General	<i>Metastrongylus pudendotectus</i>	Ont.	One case
<i>Oesophagostomum dentatum</i>	Ont., Que.	(?)	<i>Hyoststrongylus rubidus</i>	Ont.	Rare
<i>Stephanurus dentatus</i>	Man. (Winnipeg)	One specimen, no details	<i>Trichuris suis</i>	Ont., Que.	General
<i>Metastrongylus elongatus</i>	Ont., Que., N.S., P.E.I.	General	<i>Macracanthorhynchus hirudinaceus</i>	Ont.	Confined to Essex and Kent Counties, common

BIRDS (*Domestic and Game*)

Parasite	Host	Provinces	Apparent prevalence	Parasite	Host	Provinces	Apparent prevalence
TREMATODA							
<i>Streptovilella acadiae</i>	<i>Anas rubripes</i>	N.S.	Serious in 1932	<i>Notocotylus attenuatus</i>	<i>Branta canadensis</i>	Ont.	(?)
<i>Paramonostomum pseudalveatum</i>	<i>Branta canadensis</i>	N.S.	(?)	<i>Psilochasmus</i> sp.	<i>Querquedula discors</i>	Alta.	Common, 1932
CESTODA							
<i>Raillietina tetragona</i>	<i>G. gallus</i>	Ont.	(?)	<i>Hymenolepis carioca</i>	<i>G. gallus</i>	Ont.	—
<i>Raillietina cesticillus</i>	<i>G. gallus</i>	Ont.	—	<i>Hymenolepis</i> sp.	<i>Dafila acuta</i>	Alta.	(?)
				<i>Hymenolepis</i> spp.	<i>Querquedula discors</i>	Alta.	(?)
NEMATODA							
<i>Tetrameres</i> sp.	<i>Anas. dom.</i>	Ont.	(?)				

MISCELLANEOUS HOSTS*

Parasite	Host	Provinces	Apparent prevalence	Parasite	Host	Provinces	Apparent prevalence
TREMATODA							
<i>Parascocotyle longa</i>	<i>Vulpes fulva</i>	Ont., Que.	—	<i>Alaria americana</i>	<i>Vulpes fulva</i>	Que., P.E.I.	—
<i>Paragonimus</i> sp.	<i>Mustela vison</i>	Ont.	—	<i>Alaria alata</i>	<i>Vulpes fulva</i>	—	—
<i>Echinostomum</i> sp.	<i>Ondatra zibethica</i>	Alta.	—				
CESTODA							
<i>Dipylidium caninum</i>	<i>Canis familiaris</i>	Ont.	Common	<i>Taenia taeniaformis</i>	<i>Felis catus domesticus</i>	Que.	Common
	<i>Felis catus domesticus</i>	Que.		<i>Taenia pisiformis</i>	<i>Canis familiaris</i>	Que.	—
	<i>Vulpes fulva</i>	Que.		<i>Taenia hydatigena</i>	<i>Canis familiaris</i>	Que.	—
<i>Hymenolepis</i> sp.	<i>Ondatra zibethica</i>	Alta.	—				

*This heading consists of dogs, cats, foxes and miscellaneous furbearers.

MISCELLANEOUS HOSTS—*Concluded*

Parasite	Host	Provinces	Apparent prevalence	Parasite	Host	Provinces	Apparent prevalence
NEMATODA							
<i>Belascaris marginata</i>	<i>Canis familiaris</i>	Ont., Que.	Common	Physaloptera spp.	<i>Procyon lotor</i>	Que.	Common
<i>Belascaris mystax</i>	<i>Felis catus domesticus</i>	Que.	Common	Physaloptera sp.	<i>Taxidea taxus</i>	Alta.	(?)
<i>Toxascaris limbata</i>	<i>Canis familiaris</i>	Que.	Common	<i>Dioclophyme renale</i>	<i>Canis familiaris</i>	Ont., Que.	Common in Northern dogs
<i>Ascaris</i> sp.	<i>Vulpes fulva</i>	Que.	Common				
	<i>Taxidea taxus</i>	Alta.	—		<i>Mustela vison</i>	Que.	Common
<i>Ancylostoma caninum</i>	<i>Canis familiaris</i>	Ont.	Common	<i>Trichuris vulpis</i>	<i>Canis familiaris</i>	Ont., Que.	Common
	<i>Vulpes fulva</i>	Que.	(?)		<i>Vulpes fulva</i>	Que.	(?)
<i>Dochmoides stenocephala</i>	<i>Vulpes fulva</i>	Que., N.B., P.E.I.	Common	<i>Capillaria plica</i>	<i>Vulpes fulva</i>	Que.	Common
				<i>Eucoleus aerophilus</i>	<i>Vulpes fulva</i>	Que.	Common

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STUDIES ON FOOT AND ROOT ROT OF WHEAT

I. EFFECT OF AGE OF THE WHEAT PLANT UPON THE DEVELOPMENT OF FOOT AND ROOT ROT¹

By W. C. BROADFOOT²

Abstract

Studies were made to determine whether, in sterilized inoculated soil, Marquis wheat plants became more or less susceptible during the post-seedling stage to *Ophiobolus graminis*, *Helminthosporium sativum*, and *Fusarium culmorum*. While it was found that the plants in the seedling stage were more susceptible than at later stages, there was, with the technique used, no evidence that the plants become more or less susceptible during the post-seedling stage. The reasons for indefinite evidence on this important question are given. In sterilized soil, in open pot culture, inoculum of *O. graminis* was definitely more virulent when alone than when mixed singly or in combination with *H. sativum*, *F. culmorum* or *Leptosphaeria herpotrichoides*. The virulence of all pathogens mentioned decreased progressively in sterilized soil, the greatest decrease taking place during the first 40 days, after which they were only slightly pathogenic and at the end of 120 days inoculum of *O. graminis* was impotent. The virulence of inoculum when added to unsterilized soil was greatly reduced in contrast with that in sterilized soil, and after 10 days it was practically at a minimum. These results emphasize the necessity of protecting inoculated sterilized soil against contamination by other micro-organisms in critical studies made in soil. They also throw light on the much recognized difficulty of producing foot rot in the field by prepared inoculum added to such soil.

If we would understand the wheat foot-rot complex, it is important to determine if possible whether certain morphological and physiological changes, which accompany the maturation of the foot and root tissues, render the plant more or less susceptible to any or all of the pathogens concerned. To unravel the situation which exists in ordinary unsterilized soil, or under normal field conditions, where possibly all the foot-rotting fungi may be found together would be extremely difficult if not impossible. If attempted under greenhouse conditions in sterilized soil, the reaction of the plants at various ages to each organism would have to be determined, and also their reaction to various combinations of these pathogens as well.

Materials and Methods

The present study was begun in 1929 and included pathogenic cultures of *Ophiobolus graminis* Sacc., *Helminthosporium sativum* P. K. and B., *Fusarium culmorum* (W. G. Sm.) Sacc., and *Leptosphaeria herpotrichoides* de Not., all isolated from wheat plants. Each pathogene was increased separately in sterilized ground oat hulls. Whenever a pathogene was tested, either singly or in combination, a 10-gm. portion of each one was used. Pots, six inches in diameter, were filled with three parts of black loam mixed with one part of sand, and steam-sterilized for six hours at 15 lb. pressure. Elite Marquis seed was sown, covered first with or without inoculum as required, then with top soil. An equal portion of sterilized oat hulls was used in the checks to permit of a favorable comparison. The various degrees of infection are denoted as follows: 0, clean; 1, trace; 2, light —; 3, light; 4, light +; 5, medium —; 6, medium; 7, medium +; 8, heavy —; 9, heavy; 10, heavy +.

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Contribution from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada, co-operating with the University of Alberta. This paper constitutes one part of a thesis submitted May 3, 1932, to the graduate faculty of the University of Minnesota in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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In all, four different experiments were completed in connection with this general study. In the first and second experiments some pots were inoculated at seeding time, and the others at 10-day intervals thereafter until the plants neared maturity, at which time the plants were taken up and the amount of disease recorded. For the later inoculations of growing plants, the inoculum was placed about the roots, after carefully removing the upper two inches of soil from around the plants. The removed soil was then replaced. The check pots were treated in the same manner, except that sterilized non-inoculated ground oat hulls were added. Four hundred pots, six plants per pot, were used in the first experiment, each test being replicated five times; in the second there were 800 pots with six plants per pot; 128 pots, six plants per pot, in the third, each test being replicated four times; and in the fourth experiment, there were 196 pots, 15 plants per pot, each test being replicated five times, except in the case of the checks, when only two replicates were used. The chief differences in procedure among the first, second, and third experiments may be briefly stated as follows: in the first one, the plants were not taken up until mature, while in the second experiment the arrangement was such that plants of each test were harvested every 10 days. This was necessary since the first experiment indicated that if the plants remain until maturity, a uniform infection results. This obscured any real differences which might have existed at various periods. The third experiment was planned like the second, only one part was duplicated in unsterilized soil. Another difference was that *L. herpotrichoides*, which was at that time recently discovered in Alberta by Henry and Foster (6) at Camrose, and in the same season by the author (1) on winter wheat at Cowley, was included in the third experiment. The general plan of the second experiment with regard to the kind and time of inoculation and time of note taking is indicated in Table I.

Experiment 1 **Experimental Results**

The chief value of the first experiment (2) was in indicating three things: first, that the maximum degree of injury usually occurred when *O. graminis* was used alone; second, that, as indicated by Davis (3), the severity of injury decreased as the inoculations were delayed; and third, that no maximum degree of injury was produced at the different periods. Thus, the results suggested another experiment, in which periodic removal of plants should be made as well as periodic inoculations. Since the results of the first experiment were essentially the same as for the material inoculated at 10-day intervals and taken up at maturity in the second experiment, it will not be necessary to present them here. The second experiment is more extensive and hence more conclusive, giving a fairly complete picture of the progress of foot rot under greenhouse conditions.

Experiment 2

The results of the second experiment are presented in Table I. The infection rating on plants 110 days old, with regard to *F. culmorum*, *H. sativum*, and *O. graminis* (alone), inoculated at various periods are shown in Table II for comparison.

TABLE I

THE SUSCEPTIBILITY OF MARQUIS WHEAT PLANTS TO *Fusarium culmorum*, *Helminthosporium sativum*, AND *Ophiobolus graminis*, ASCERTAINED AT 10-DAY INTERVALS FROM SEEDLING STAGE TO MATURITY. THE INOCULUM WAS APPLIED SINGLY AND IN COMBINATION AT SEEDING AND AFTER 10-DAY INTERVALS THEREAFTER. THE INFECTION RATING IS GIVEN BELOW NUMERICALLY^a

Culture ^b	Days after planting of inoculating (numerator) and of harvesting (denominator)															
	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160
1F	0.4	1.2	1.3	0.8	1.6	0.6	2.2	0.7	2.6	0.6	1.6	2.5	0.3	0.1	0.6	1.2
2H	1.6	0.2	1.9	2.0	1.6	0.7	0.2	0.9	0.7	2.0	2.9	0.2	0.2	1.0	0.5	1.9
3O	1.2	0.1	2.2	3.2	6.6	6.0	5.6	8.7	8.6	8.4	8.8	0.0	0.5	0.9	3.5	0.6
4F+H	1.5	0.3	1.5	0.8	1.5	0.8	0.6	1.1	2.4	0.8	1.7	0.7	0.4	0.4	0.7	1.3
5F+O	0.8	0.0	1.7	2.5	3.8	5.3	7.3	1.5	7.0	8.9	8.4	0.1	0.2	0.6	1.5	2.3
6H+O	1.4	0.3	1.6	3.0	4.4	4.6	5.3	6.0	5.6	7.3	8.0	0.1	1.2	0.5	1.9	1.8
7H+O+F	1.2	1.3	1.5	2.5	4.1	1.6	1.5	7.6	7.6	7.4	6.9	0.0	0.9	0.6	2.3	3.7
8 Check	0.0	0.1	0.2	0.3	0.0	0.2	0.1	0.3	0.1	0.8	0.5	0.5	0.2	0.4	0.2	0.2

TABLE I—continued

Culture ^b	Days after planting of inoculating (numerator) and of harvesting (denominator)															
	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180
1F	0.5	1.0	0.7	0.9	3.2	1.2	1.2	1.6	1.4	1.9	1.3	1.7	1.4	3.2	2.0	1.9
2H	0.9	0.7	0.8	0.7	1.4	1.7	1.0	4.8	7.3	6.8	1.5	0.7	1.4	1.0	1.2	1.0
3O	0.9	0.9	0.9	1.4	1.2	1.2	1.2	1.2	1.2	1.3	1.5	0.7	1.4	1.0	1.7	1.5
4F+H	0.2	1.4	1.0	1.5	2.9	1.6	4.0	5.8	7.4	1.1	1.0	1.7	3.0	2.0	2.5	3.3
5F+O	0.6	0.9	1.9	1.5	1.4	4.4	3.0	5.7	6.8	1.1	1.0	1.5	1.7	2.0	3.8	5.9
6H+O	1.2	0.7	1.3	1.5	4.3	4.9	6.0	6.0	6.2	1.1	0.7	1.0	1.7	2.0	1.8	4.3
7H+O+F	0.2	0.2	0.1	0.3	0.4	1.4	0.7	0.3	0.5	0.5	0.2	0.2	0.1	0.1	0.3	0.3
8 Check	0.2	0.2	0.1	0.3	0.4	1.4	0.7	0.3	0.5	0.5	0.2	0.2	0.1	0.1	0.3	0.3

TABLE I—continued

Culture ^b	Days after planting of inoculating (numerator) and of harvesting (denominator)															
	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200
1F	0.7	1.2	1.2	1.1	1.5	2.0	0.5	0.5	0.4	0.2	0.5	1.0	0.6	0.3	0.4	0.2
2H	0.0	0.2	1.1	0.5	0.9	0.4	2.1	0.1	0.2	2.0	0.4	0.3	0.5	0.1	0.4	0.4
3O	0.3	0.5	0.7	1.5	1.0	1.0	2.1	0.1	0.2	0.5	0.8	0.6	0.3	0.2	0.0	0.4
4F+H	0.5	0.4	0.4	2.3	1.3	1.0	0.3	0.7	0.8	0.8	0.2	0.5	0.2	0.2	0.2	0.4
5F+O	0.2	0.3	0.7	0.6	0.5	1.1	1.1	1.0	0.6	1.4	0.2	0.4	0.6	0.2	0.1	0.5
6H+O	0.8	2.0	1.1	2.1	2.1	1.2	1.4	0.1	0.6	1.1	0.8	0.7	0.2	0.2	0.4	0.2
7H+O+F	0.0	0.2	0.2	0.5	2.2	2.2	1.1	0.2	0.2	0.2	0.3	0.3	0.3	0.1	0.0	0.3
8 Check	0.0	0.2	0.2	0.5	2.2	2.2	1.1	0.2	0.2	0.2	0.3	0.3	0.3	0.1	0.0	0.3

^a Infection rating—0, clean; 1, trace; 2, light; 3, light; 4, light; 5, medium; 6, medium; 7, medium; 8, heavy; 9, heavy; 10, heavy.

^b Culture—F, *Fusarium culmorum*; H, *Helminthosporium sativum*; O, *Ophiobolus graminis*. In each case a 10-day sample of the respective culture was added.

TABLE II
INFECTION RATING ON PLANTS 110 DAYS OLD, INOCULATED AT VARIOUS PERIODS

Inoculation	<i>F. culmorum</i>	<i>H. sativum</i>	<i>O. graminis</i>
At planting	2.5	2.9	.8.8
10 days	2.0	2.2	7.3
20 days	3.2	1.4	6.8
30 days	1.1	0.3	7.6
40 days	1.3	1.0	2.8
50 days	0.5	0.3	2.1
60 days	0.6	0.5	0.8
70 days	0.3	0.4	0.5
80 days	0.4	0.4	0.4
90 days	0.3	0.4	0.4

Obviously *O. graminis* was much more virulent than either *F. culmorum* or *H. sativum*. Also inoculations made up to 40 days were in general fairly virulent, and those made later were relatively ineffective, particularly those having *F. culmorum* or *H. sativum*. The results in Table I show that in about 40 days the infection decreased as much in those pots where either *F. culmorum* or *H. sativum* were mixed with *O. graminis* as it did in those pots in which *O. graminis* was used alone.

Robertson (8), who carried on histological studies in connection with this problem, found a marked increase in the amount of lignified tissue in the foot of Marquis wheat plants taking place with advancing age. He concluded that the thickening of the walls and the increase in lignification or other chemical changes accompanying maturation of the root may at times retard the progress of foot-rotting fungi. He found that the increase in lignification occurred chiefly in the xylem, the pericycle, the fibres of the vascular bundle sheaths, and in the sub-epidermal layers of the cortex of the crown roots. The cells of the latter became much thickened with advancing age. This increase in lignification reached its maximum in about 40 days, which was coincident with a marked falling off in infection of wheat plants by *O. graminis*.

However, in addition to these changes, which occur in the host, there may also be certain environmental factors, *i.e.*, the association of other micro-organisms, which influence the ability of the parasite to infect wheat plants when they have been inoculated 50 days after planting time. The time it takes *O. graminis* to successfully establish itself in the host as it becomes older must not be minimized. Also there is the fact that the sterilized soil gradually becomes populated with foreign micro-organisms under greenhouse conditions, and these more or less suppress the virulence of the pathogene. Sanford and Broadfoot (9) have already shown that a wide range of soil-inhabiting bacteria and fungi and their filtrates more or less suppress the virulence of *O. graminis*, depending on the particular micro-organism involved. Fellows (4) states that "the inoculum of *O. graminis* soon loses its virulence when added to uninfested soil." Further, the results given in Table I show that when inoculations were made at time of planting, symptoms of *O. graminis* were evident on the primary roots 10 days after inoculation, and on the

secondary roots after 20 days. However, the infection rating at the end of 10 days is rather low, but naturally this increases rapidly thereafter, becoming pronounced at 30 days and later. When the inoculum was applied 10 days, 20 days, or later, after planting, there was a corresponding decrease in development of disease. That is to say, in addition to chemical and morphological changes, which occur as the foot and root parts of wheat age, tending perhaps to make it more resistant, one must make allowance also for factors which increase or decrease the virulence of the pathogene at any stage of growth. Lüttke (7) considers it probable that the greater susceptibility of young wheat plants to *O. graminis* and *F. culmorum* is due to the part played by certain enzymes in the decomposition of the polymeric carbohydrates, the success of which is dependent on coincidence between the development of the fungus and that of the host. Whether the greater susceptibility of the wheat plant at this period can be explained entirely on a chemical or on a morphological basis is an interesting question.

With regard to the effect of mixing *F. culmorum* or *H. sativum* with *O. graminis*, or of mixing all three together, on the infection produced, the results in Table I, which are shown in Table III for comparison, are interesting. The plants were inoculated at various periods, and all were examined at maturity.

TABLE III

EFFECT OF MIXING *F. culmorum* OR *H. sativum* WITH *O. graminis*, OR OF ALL THREE TOGETHER, ON THE INFECTION PRODUCED

Inoculation	<i>O. graminis</i>	<i>F. culmorum</i> + <i>O. graminis</i>	<i>H. sativum</i> + <i>O. graminis</i>	<i>F. culmorum</i> + <i>H. sativum</i> + <i>O. graminis</i>
At planting	8.8	8.4	8.0	6.9
10 days	7.3	4.5	4.2	6.2
20 days	6.8	7.4	6.8	6.2
30 days	7.6	6.3	5.9	5.9
40 days	2.8	5.0	5.5	5.4
50 days	2.1	2.1	1.1	1.4
60 days	0.8	0.8	1.4	0.8
70 days	0.5	0.7	0.3	0.4
80 days	0.4	0.4	0.8	0.4
90 days	0.4	0.5	0.2	0.4

Apparently the virulence of *O. graminis* was somewhat reduced by either or both *F. culmorum* and *H. sativum*. The minor exceptions which occurred, and which were probably caused by contaminations, do not appear to be of sufficient importance to alter this conclusion. It must be remembered that the experiment was begun with sterilized soil, using all available precautions to avoid unwanted contaminations. However, these occurred, because the soil had to be removed from around the roots for the inoculations made after planting time.

Experiment 3

This experiment differed from Experiment 2 mainly in having one part on sterilized soil and the other on unsterilized soil. The results are contained in Table V. The infection ratings for *O. graminis* alone and with one or more

of *F. culmorum*, *H. sativum*, and *L. herpotrichoides* in the two soil series are listed in Table IV for comparison. The inoculum was applied at planting time, and the plants were taken up at maturity.

TABLE IV
INFECTION RATINGS FOR *O. graminis* ALONE AND WITH ONE OR MORE OF *F. culmorum*, *H. sativum*, AND *L. herpotrichoides* IN THE TWO SOIL SERIES

	Infection rating	
	Sterilized soil	Unsterilized soil
3. <i>O. graminis</i>	6.6	3.4
5. <i>O. graminis</i> + <i>F. culmorum</i>	3.6	1.0
6. <i>O. graminis</i> + <i>H. sativum</i>	3.8	2.3
7. <i>O. graminis</i> + <i>H. sativum</i> + <i>F. culmorum</i>	3.0	2.3
12. <i>O. graminis</i> + <i>L. herpotrichoides</i>	3.5	1.2
14. <i>O. graminis</i> + <i>L. herpotrichoides</i> + <i>F. culmorum</i>	4.6	4.4
15. <i>O. graminis</i> + <i>L. herpotrichoides</i> + <i>H. sativum</i>	4.6	1.7
16. <i>O. graminis</i> + <i>L. herpotrichoides</i> + <i>H. sativum</i> + <i>F. culmorum</i>	1.5	2.4

TABLE V
THE EFFECT OF SINGLE, DOUBLE, TRIPLE, AND QUADRUPLICATE COMBINATION INOCULATIONS WITH *Fusarium culmorum*, *Helminthosporium sativum*, *Ophiobolus graminis*, AND *Leptosphaeria herpotrichoides* ON MARQUIS WHEAT IN STERILIZED AND UNSTERILIZED SOIL. INOCULUM WAS APPLIED AT PLANTING TIME AND PLANTS WERE TAKEN UP AT MATURITY. A NUMERICAL INFECTION RATING IS GIVEN

Culture ^b	Sterilized soil		Unsterilized soil	
	Height in cm. ^a	Infection rating ^a	Height in cm. ^a	Infection rating ^a
1. F	62.3	2.9	57.7	1.4
2. H	59.8	3.3	62.3	0.8
3. O	63.6	6.6	59.5	3.4
4. F + H	50.2	3.7	53.2	2.7
5. F + O	62.9	3.6	62.3	1.0
6. H + O	56.0	3.8	60.2	2.3
7. H + O + F	61.4	3.0	57.3	2.3
8. Check	66.3	1.0	66.5	1.2
9. L	66.7	3.1	60.4	1.1
10. F + L	66.6	2.8	58.8	1.2
11. H + L	65.8	3.0	55.4	1.6
12. L + O	57.6	3.5	58.0	1.2
13. F + H + L	60.3	4.1	59.8	1.3
14. F + L + O	66.2	4.6	46.6	4.4
15. H + L + O	62.2	4.6	60.9	1.7
16. F + H + L + O	61.7	1.5	53.8	2.4

^a Average of four replicates.

^b Culture. F, *Fusarium culmorum*; H, *Helminthosporium sativum*; O, *Ophiobolus graminis*; and L, *Leptosphaeria herpotrichoides*. In each case a 10-gram sample of each culture was added at seed level.

In general, *O. graminis* was more virulent when alone than when mixed in various combinations with the other pathogens mentioned, whether in

sterilized soil or unsterilized soil. Also, the pathogenicity of *O. graminis* was reduced more in unsterilized soil than in the sterilized soil. This was a common observation. Since this paper was first presented, a similar observation has been made by Henry (5). An explanation cannot be offered now for treatment No. 14 in the unsterilized soil, where the infection was greater than in treatment No. 3 (*O. graminis* alone).

Experiment 4

This experiment was designed to show the differences in severity of *O. graminis* on wheat produced by inoculating the soil at planting time, and subsequently at 10-day intervals, in sterilized and in unsterilized soil. In one series (Series I) all the pots were inoculated and then the seed was sown at 10-day intervals. In the other part (Series II) the pots were prepared as in the first series and kept watered on the bench to be inoculated and seeded simultaneously at 10-day intervals. In this way it was hoped to obtain the effect of the microflora, which developed in either sterilized or unsterilized soil in both series, on the inoculum added (1) at the beginning, and (2) at intervals subsequently. The results are given in Table VI and are graphically represented in Fig. 1. The plants were taken up 30 days after planting time in each case.

Considering first Series I, where the inoculum was added July 30 in sterilized soil, it will be observed that the infection rating decreases from 6.5 to 4.8, 4.1, 3.0 and finally to 1.7 after 10, 20, 30, and 60 days, respectively.

Contrast these results with those where the inoculum and seed were planted together in soil originally sterilized. Here the infection decreased from 7.4 to 1.7, 0.8, 1.3, and finally to 0.8, after 10, 20, 30, and 60 days, respectively. The decrease in virulence of *O. graminis* in the pots of sterilized soil, exposed to ordinary contamination from watering and from the air, after 10 days, was approximately equal to that which occurred after 50 days in the first

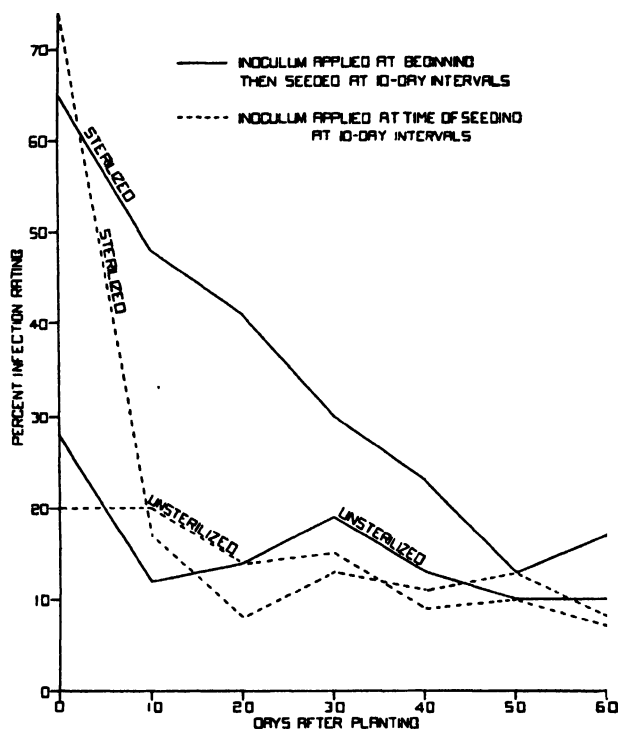


FIG. 1. The differences in severity of *Ophiobolus graminis* on Marquis wheat plants obtained by periodic applications of the pathogene to an initially sterilized soil series and to an unsterilized soil series.

series, when the inoculum had been added to the soil at the beginning. Apparently in the *absence* of inoculum, flora antagonistic to *O. graminis* quickly increased in the sterilized soil, but this was delayed somewhat in the *presence* of the inoculum. As in the previous experiment, a low infection rating was obtained in the unsterilized soil. In fact it is interesting to note, in Table VI, that the initial infection in the unsterilized soil was greater than

TABLE VI

THE EFFECT OF INOCULATING WITH *Ophiobolus graminis* AT PLANTING TIME, AND AT 10-DAY INTERVALS, ON MARQUIS WHEAT SOWN AT 10-DAY INTERVALS IN STERILIZED AND UNSTERILIZED SOIL. PLANTS WERE TAKEN UP 30 DAYS AFTER PLANTING TIME IN EACH CASE*. NUMERICAL INFECTION RATING IS GIVEN

Date of planting	Series I. Inoculum of <i>O. graminis</i> applied July 30 to all inoculated pots. Sown at 10-day intervals				Series II. Inoculum of <i>O. graminis</i> applied at 10-day intervals, starting July 30. Sown at 10-day intervals			
	Sterilized soil		Unsterilized soil		Sterilized soil		Unsterilized soil	
	Height ^a	Infection rating ^a	Height ^a	Infection rating ^a	Height ^a	Infection rating ^a	Height ^a	Infection rating ^a
July 30 Check	46.3	6.5	52.4	2.8	51.0	7.4	57.7	2.0
	49.9	0.3	49.6	0.8	55.1	0.3	56.2	1.0
Aug. 10 Check	51.4	4.8	47.8	1.2	56.2	1.7	47.7	2.0
	54.5	0.1	55.2	0.3	54.8	0.1	48.3	0.5
Aug. 20 Check	50.9	4.1	55.1	1.4	53.0	0.8	51.5	1.4
	50.5	0.2	44.8	1.1	52.5	0.6	44.4	1.0
Aug. 30 Check	50.1	3.0	46.2	1.9	54.2	1.3	45.1	1.5
	48.9	0.4	37.9	0.8	49.2	0.3	41.3	0.9
Sept. 10 Check	50.1	2.5	49.4	1.3	52.5	1.1	52.4	0.9
	46.5	0.2	41.1	0.8	53.7	0.5	43.1	0.8
Sept. 20 Check	51.2	1.3	51.0	1.0	51.3	1.3	47.5	1.0
	48.6	0.3	47.1	0.4	46.1	0.2	44.7	0.4
Sept. 30 Check	46.8	1.7	47.6	1.0	43.9	0.8	49.3	0.7
	46.8	0.3	47.1	1.2	47.5	0.3	50.8	0.4

* Average of five replicates for the inoculated pots and only two for the checks.

it was after subsequent inoculations or plantings. Apparently the initial flora of the soil was ample in either case to suppress the pathogenicity of *O. graminis* at first, and later to reduce it further, where the pathogene either disappeared or became practically impotent. This fact possibly explains why inocula of the foot-rotting fungi are relatively ineffective when added to field soil.

A further illustration of how the virulence of *O. graminis* becomes vitiated in sterilized soil under ordinary greenhouse conditions may be cited now. Ten days after the plants were removed from the pots to which *O. graminis* had been added at planting time, and subsequently at 10-day intervals in

Experiment 2, they were re-seeded with Marquis wheat. These plants were examined at maturity. The maximum original infection was about 8.8 (Table I). However, when the plants of the second crop in the same soil were harvested, the virulence of *O. graminis* had diminished to practically zero. The plants were remarkably clean. The time from when the inoculum was placed in the soil to the time of re-seeding varied from 20 up to 120 days, and in all cases, 120 days from the time the soil was originally sterilized.

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PERENNIAL CANKER OF APPLE TREES¹

BY H. R. McLARTY²

Abstract

In 1925 a new disease of apple trees was described by Zeller and Childs and named Perennial Canker (*Gloeosporium perennans*). The results of the various investigators who have been working on the problem indicate a considerable difference of opinion as to the various factors responsible for the production and spread of the disease. This paper presents the results of a study of these factors and an interpretation of the part that each plays in the production of the disease. It is shown that its occurrence is entirely dependent on three factors; the annual inoculation of the host with the causal organism during the late summer and autumn; the presence of the woolly apple aphid which carries out this inoculation; and the exposure of the host after inoculation to periods of low temperature. Other factors such as winter injury, time of pruning and precipitation, are contributory to its severity, but not essential for its general occurrence.

Introduction

Perennial canker is a new disease attacking apple trees in most of the fruit-growing sections of Oregon, Washington and British Columbia. It was first recorded in the literature in 1925, though it is now quite apparent that the disease had already been present for several years. Its occurrence during that time was not unnoticed but it was thought to be a phase of the disease caused by the northwestern anthracnose fungus *Pezizula malicorticis* (Jackson) Nannf. (23).

Since 1925 various investigators have given considerable attention to the cause and cure of the trouble. In August, 1925, Zeller and Childs issued a paper (27) and a bulletin (26) which described in fair detail the general symptoms of the canker produced in the tree, and the rot in the fruit. They described also the characteristics of the fungus which they isolated from the canker. They were of the opinion at that time that the fungus producing the canker was perennial in the tissues of the host; consequently they classified the fungus as *Gloeosporium perennans* and named the disease "perennial canker." They reported that observational evidence indicated that the extent of infection was greater after severe winters than after mild ones. They did not bring forth any evidence, however, that the fungus was pathogenic in artificial inoculations.

In the same year Fisher (6) presented a paper on a new rot of apple fruit. This apple rot which he called bull's eye rot was described and distinguished from the rot caused by northwestern anthracnose *P. malicorticis*. He also connected the rot with the canker in the tree and believed both to be produced by the same fungus.

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The writer, in his publications for 1925 (13, 14) described the disease, and showed that the fungus isolated from these cankers and grown in pure culture is capable, when inoculated into apple limbs, of producing infection similar to that occurring in the original cankers. From such infection, the fungus in pure culture was re-isolated. The extensive saprophytic nature of the fungus was shown by the fact that the writer obtained viable spores from bark that had been dead for two years, from which cultures were grown and successful inoculations made.

During the following year, publications appeared by Fisher (7) and the writer (15). The former, working with the rot in the fruit, describes the origin of the rot as being due to spores from cankers being washed to the fruit by rain. The latter suggests that the fungus in the host tissues is annual in nature and not perennial. He found that cankers which he had produced by artificial inoculation healed over normally, although a year later the fungus could be isolated from the affected heartwood. He showed that the fungus becomes closed-in through the normal growth process of the summer season, and that it cannot, of itself, re-infect the phloem tissue or the newly deposited xylem tissue. He concluded, therefore, that the pathogenic phase of the fungus is strictly annual, and that there must be present some inoculating agency in the orchard if there is to be a re-infection. By marking the position of woolly aphid *Schizoneura lanigera* Hausmann, on cankers during the fall of 1925, and noting the subsequent occurrence of infection in the spring of 1926, he concluded that this aphid was the agent inducing all new infection in established cankers. In this report, results are given also of artificial inoculations indicating that he was successful in inducing infection readily during October, November and December, but from May to September he experienced difficulty in doing so.

An anonymous article in Better Fruit (28) in 1927, records the results of Childs' work at Hood River. This work seems to verify that of the writer with regard to the necessity of having woolly aphides present for the spread of infection. Though the author of the article (28) believed that aphides were definitely responsible for the establishment of new cankers, he does not show clearly whether or not he considered them to be responsible for all annual enlargement of an already established canker. He did show however that, by cutting out diseased tissue in old cankers and excluding aphides, further enlargement was prevented.

In the same year, the writer (16) drew attention to the spread of this aphid in the orchard through the agency of the wind. Limbs painted with pine tar served as a trap on which were collected, in a very short time, large numbers of the very young nymphs. Their equal distribution over the whole painted area led him to believe that they could be so distributed only by the wind, since they could not have crawled over the sticky surface.

In 1928, Fisher and Reeves (8) reported that water-borne spores may induce infection of tissue which has not previously been wounded or suffered from aphid attack. These infections were stated to be confined to the outer

cortex tissue. They believed also that the severity of the disease is directly correlated with rainfall, the disease being more severe in areas of greater precipitation. Though they recorded the annual mean temperatures for certain districts, they did not suggest that winter temperatures had any influence on the amount of spread. The influence of aphides was suggested, but they believed that effectual control of the canker involved cutting out of all infected tissue, followed by application of a disinfectant wound dressing.

In the same year, the writer (17, 18, 19) presented further evidence on the relation of aphides to spread of infection. In May, 1927, he wrapped a number of cankers in which had just occurred a normal spread of infection; no treatment of the canker was given except the wrapping. In 86 of these cankers there were no aphides present at the time of wrapping, and in 44 aphides were present. These cankers were undisturbed until May, 1928, when the wrappings were removed. It was found that the 86 cankers which had been aphid-free showed absolutely no enlargement at the end of the year period, and in all 44 cankers where aphides had been present there was new infection in the phloem with resultant canker enlargement. In this report also, a description of the fruit rot is given as it occurs on nine varieties of apples.

In 1929 articles appeared by White (25), Childs (1, 2), Fisher and Reeves (9), and the writer (20, 21). White gives no experimental evidence, but believes that fertilizers might be of benefit in control, through the building up of the vitality of the tree. Childs reviewed the work conducted in the Hood River Valley from 1925-1928. He believed, first, that perennial canker is due to a wound parasite, apparently incapable of entering sound uninjured tissue; second, that the fungus producing the canker is not truly perennial; third, that the woolly aphid is definitely associated with canker infection; fourth, that canker infection usually follows severe late aphid infestations; fifth, that extent of canker advance is to a large degree governed by winter temperatures; sixth, that aphides are instrumental in producing cracking of the callus and so permitting entrance of the organism; seventh, that infection from pruning is more severe when these operations are carried on in December, than in March. No beneficial results were obtained with spraying, and he considered that control must be through surgery, wound painting and spraying.

Fisher and Reeves brought forth further evidence to substantiate their claims concerning water-borne spores as sources of fruit infection, and they further claimed that rain-washed spores are able to induce infections not only in the outer cortex layers as formerly stated (8), but that such infections also often reached the cambium. They recorded also the enlarging of established cankers without the influence of any aphid attack. They considered that the advance of the fungus was preceded by a killing of the surrounding tissues, presumably by enzymic action. On the basis of their findings they recommended control measures consisting of spraying and surgical treatment to remove all diseased tissue.

The writer carried out some experimental work on the influence of frost on the spread of canker. By erecting a wooden framework around the lower portion of a tree and filling the frame with sawdust, a number of cankers were protected from exposure to freezing temperatures during the following winter. The tree used was both severely affected with cankers and infested with aphides. These results indicated that where no frost reached the tissue, there was no spread in the cankers. In the unprotected cankers on the same tree, normal spread occurred.

During 1930 Cooley and Miller (4) in making artificial inoculations verified the writer's findings as to the highest percentage of successful inoculations occurring from those made in the fall. They believed that normal canker spread was due largely to winter injury, inasmuch as primary killing often resulted from the effect of winter temperatures and this was followed by fungous infection. They made no reference to woolly aphides as having any relation to the disease. Later, Crenshaw and Cooley (5) experimenting with the effects of winter injury on callus tissues, reported being able to kill the callus tissue by means of artificial freezing; aphid-attacked calluses were killed more easily than calluses free from aphides, and a callus around a canker was killed more easily than a normal callus.

Güssow (11) reported the disease as being most severe on those varieties subject to winter injury. He concluded that winter injury was the predisposing factor; next to winter injury he considered woolly aphid of importance as the carrier of the disease.

In his 1930 report, the writer (22) presented the results of a tree-to-tree survey of the prevalence of the disease throughout the Okanagan fruit section of British Columbia. In a brief statement, he also reported that the exclusion of aphides from cankers from July to the end of the season prevented any spread of infection in those cankers.

In February, 1932, Cooley summed up the work in Oregon for several years previous. He believes that canker infections usually take place in winter-injured calluses. Occasionally fresh pruning wounds may be affected. He considers vigorous trees to be less susceptible to winter injury, and therefore to canker infection. The presence of woolly aphides in a callus increases its susceptibility to winter injury and thus is a secondary agent increasing the amount of infection. For control he suggests a wax treatment to keep out aphides, and the delaying of pruning operations until just before growth starts in the spring.

It is quite apparent from this review of the literature that there is as yet much diversity of opinion as to the influence which any of several varying factors have on the production of the disease. The author's purpose in this paper is to present a description of this disease and an account of those causal factors which he considers are responsible for its occurrence.

The Disease

1. *Characteristics of the Canker in the Tree*

The disease produces a canker in the tree (Fig. 1) and a rot of the fruit. Cankers originate chiefly around pruning wounds (Fig. 2). The disease is commonly characterized by an excessive amount of dead callus or broken bark around such a wound (Fig. 3) and the presence on the dead bark of a number of pimples which have broken open disclosing grayish black areas beneath; or where the bark has fallen off, by the exposure of a number of concentric rings of wood tissue surrounding a sunken centre (Fig. 4).

Throughout the summer and fall, however, all cankers appear to be encircled with a healthy healing callus and all indications would suggest that a normal healing is then in progress. It is just before spring growth activity in the tree occurs that new trouble in the cankers develops. At this time, the callus in part or over its whole circumference turns brown, and in about two or three weeks' time is killed back a distance of $\frac{1}{4}$, $\frac{1}{2}$, 1 in., or even more. The disease is not long active, however, for by the time the leaves are well out on the trees the advance of infection into healthy tissue is found to have been checked and a crack appears between the recently killed area and the healthy bark. The formation of a healing callus along the margin of the crack begins again and at the end of the summer the wound appears much as it had a year previously except for the enlargement in the season just passed and the presence of one more ring of dead wood callus.

The concentric rings of a canker seemingly overlapping each other and encircling a deep centre arise from the fact that the period of infection spread occurs in the spring and ceases before the summer's wood tissue is laid down. Old cankers, therefore, have many rings and the age of a canker in years can in general be reckoned as corresponding to the number of these rings. Many exceptions to this occur where infection occurs on only one side or on spots of a callus in one year, and on the other side or in other areas the year following. In such a case it is difficult to tell in subsequent years whether the sections of rings occurred simultaneously or in succession.

Cankers occur on any part of the trunk and limbs of the tree although old trees exhibit trunk cankers more rarely. Serious infections commonly show several hundred cankers on 15- or 20-year-old trees, and the number of these coupled with the size they attain interfere so seriously with the conducting tissues of the trees that the latter become devitalized. Small twigs are frequently girdled and killed in one or two seasons and stand out from the foliage. When an advanced stage of the disease is reached, whole limbs become girdled and trees succumb abruptly.

Another type of canker is associated with the disease which is known as button canker. This does not originate around pruning wounds but around lenticels on uninjured tissue. These infections do not generally penetrate into the cambium and as they complete their spread in one season, they slough off and unless infested with woolly aphides never produce a canker showing successive enlargements.



FIG. 2. A pruning wound showing the extent of first year's infection.



FIG. 1. A Yellow Newtown tree severely affected with perennial canker. Note the coalescing of the cankers on the upper portion of the central leader.



3.

FIG. 3. A four-year-old canker showing the killed bark still in position.

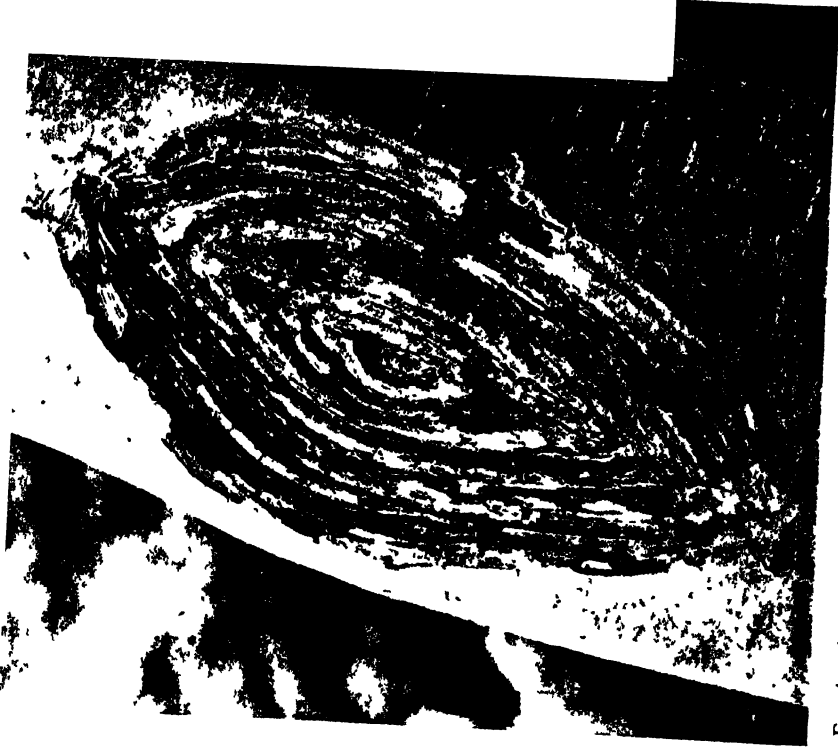


FIG. 4. An old canker with depth centre showing a number of rings of wood callus.

2. Characteristics of Rot in the Fruit

The disease also produces a rot in the fruit, and all varieties tested have shown susceptibility. Where precipitation is high during the picking season, losses sustained from this source are very considerable. The spores are washed to the fruit by rain and originate a rot chiefly around the stem end but frequently on other portions of the ripe fruit. The rot is slow in spreading, is deep brown in color around the outer edge, shading to a slightly lighter brown in the centre and beneath the surface. The infected tissue is somewhat spongy in texture, is fairly firm and usually somewhat shrunken. Toward the centre of this area the fruiting bodies of the causal fungus frequently appear as grayish white pustules breaking through the surface. At times these are in concentric rings but are not characteristically so.

The Causal Factors of the Disease

As noted above, there exists a certain diversity of opinion among the various investigators working on this problem as to the importance that several factors have in the production of this disease. There is presented, therefore, a discussion of these factors and an interpretation of the part that each plays in its production.

It is our opinion that there are three factors which must be associated before the disease can be produced. In the absence of one or more of these no disease would occur. These three limiting factors are: first, infection of the tissue with the fungus *G. perennans*; second, annual infestation in the orchard during the late summer and fall of the apple woolly aphis on callus tissue; and third, the exposure of the apple tree itself to periods of low temperatures during the winter months. In addition there are several contributory factors which serve to augment the severity of the disease. These are winter injury, precipitation, systems of pruning, variety, cultural practices, and vigor of tree.

I. Saprophytism and Parasitism of *Gloeosporium Perennans*

Saprophytic Stage

G. perennans in its life history exhibits both a parasitic and a saprophytic phase. As a saprophyte it is capable of completing a life cycle which will assure its continuation in nature indefinitely and quite independently of any parasitic phase. It grows and sporulates on several media including dead alfalfa and sweet clover stems. The cycle is simple, consisting of the production of a mycelium from a germinating spore and the production of spores from the ends of the mycelia.

It exhibits a saprophytic phase also in apple tree tissue. Upon completion of a parasitic stage in the phloem, it develops fruiting bodies characteristic of the *Gloeosporium* type on the tissue recently killed. The acervuli appear about a month after the tissue is dead and spores are liberated from these.

In some cases several crops of spores are apparently liberated from the same acervulus for a period of over a year (Fig. 5). In addition to this recurrent spore production by original acervuli, new acervuli continue to arise and liberate spores, thus assuring a continuous and ample production

of viable spores throughout the season. Germination tests have shown that such spores are readily obtainable from fruiting bodies on wood killed two years previously.

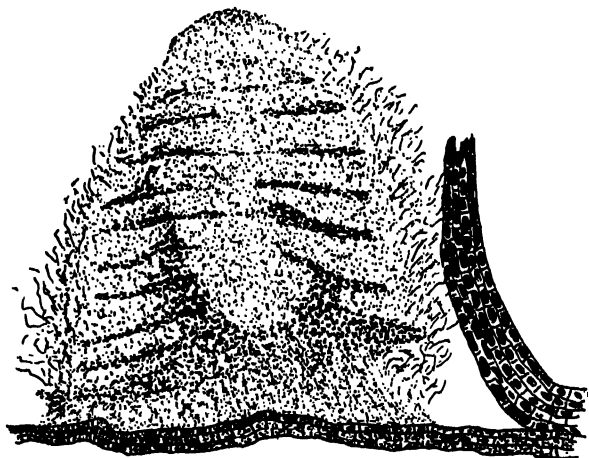


FIG. 5. A mature acervulus showing the evidence of having produced several crops of spores.

Parasitic Stage

As a parasite the fungus attacks ripe apple fruit and both the phloem and xylem tissues of the tree. Except in the case of button cankers, referred to above, the parasitic phase can be induced only through some inoculating agency. In the xylem the attack is characterized by a blackening of the tissue. In artificial inoculations this discoloration occurs as a narrow black streak shading to brown at the tip and running longitudinally for as much as two feet from the point of inoculation, but penetrating only to sap wood tissue of one to three years of age. Infection from aphid attack shows some blackening of heartwood in the vicinity of the area fed upon, but the occurrence of streaks in the xylem is only occasional.

Infection in the xylem may be induced artificially at almost any season of the year but the period of spread is limited to one season's activity. No spread occurs from the xylem into the cambium and phloem, and although isolations have been made from the blackened area showing the fungus to be alive for as much as 18 months after the original inoculation, there is no increase in the extent of spread after the first season's activity.

In the phloem, the time during which infection advances is quite definitely limited to the late winter and early spring. The inoculations which induce these infections occur however during the late summer and fall. The first evidences of infection appear in the early winter. In January a blackening of the phloem indicates the beginning of infection which advances rapidly first through the sieve tubes and companion cells but which eventually impregnates the whole tissue. By March 15 the extent of spread has been found in artificial inoculations to be as much as five inches from the point of inoculation. Shortly after this the spread is checked and by the time the buds are showing a green tip, the parasitic stage is completed. The tree

forms a healthy callus and the subsequent development of the fungus is saprophytic. It is incapable of renewing its parasitic phase unless it is re-inoculated into the host tissue.

The parasitic development of the fungus in phloem tissue is also dependent on other factors besides that of an inoculating agent. It has been found that there exists a definite period of the year during which successful inoculations can be made. In artificial inoculations this period occurs during the months of October, November and December. Inoculations induced through the influence of aphid attack occur from the early part of August. Until this time of the season, neither artificial inoculations in the open nor aphid infestation will induce any resultant infection.

A third factor governing the parasitic attack of this fungus is that the host must first be brought into the correct physiological condition. When inoculations are made either artificially or through aphid attack, infection into the surrounding uninjured tissue does not immediately occur. The killing of this tissue takes place only after it has been exposed to freezing temperatures. The technique used in inoculation also appears to influence somewhat the amount of resultant infection. Any technique that will assure the killing of, or severe injury to a large number of cells at the point of inoculation, favors the attack of the fungus. Long aphid infestation which greatly weakens the tissue, or in artificial inoculation, the cutting through to the cambium several times with the scalpel, assures a stronger resultant infection. It would appear that in the period of incubation the fungus strongly establishes itself in this injured tissue and from this point it can initiate a stronger parasitic attack.

Characteristics of the Fungus in the Host

In examining the fungus in the host tissue, attempts to make sections by means of the commonly recommended technique of embedding and sectioning were unsuccessful. A technique was developed which was satisfactory for phloem and fruit tissues, but only moderately so for the xylem. It consisted of treating the phloem and fruit tissues with a macerating solution (10) of 1% sodium carbonate or ammonium oxalate to dissolve out the pectin from the middle lamella and allow separation of the cells. The material was placed, without previous killing, in the solution and held at 80 to 90° C. for two to four hours. It was then carefully removed to a watch glass, rinsed in water and stained in Pianese IIIB (24) at 80 to 90° C. for a half-hour. The material was then washed by flooding and removed in small quantities to a glass slide where it was teased out in water. A drop of glycerol was added and mixed well with the water and a cover slip was placed on it. The whole was allowed to stand two weeks, after which the edges of the cover slip were sealed with Canada balsam. For xylem tissue the procedure was the same except for the substitution of a macerating solution (12) made up as follows: chromic acid, 5 gm.; nitric acid, 5 cc.; water, 100 cc. With the xylem, however, it was found that the solution necessary to dissolve lignified tissue dissolved some of the mycelium.

By the above method of examination of the xylem, mycelium was detected only in the tracheae. Usually one to four strands were present in a single vessel, these occurring in characteristically straight strands having few side branches.

In the phloem the mycelium penetrates through all the tissues. It is both inter- and intra-cellular. Most rapid advance is in the sieve tubes, later extending to the companion cells and cortical tissue. In the sieve tubes, as in the tracheae, not more than four or five long slightly branched strands occur in a single tube. In the companion cells and cortical tissue the fungus is inter-cellular, much branched and varying widely in diameter within short distances. No haustoria could be found. Generally many vacuoles appeared in the cytoplasm and rendered the septation of the fungus somewhat indistinct.

In the fruit flesh, mycelium was abundant, penetrating the cells and crossing in every direction to form a net work.

The acervuli appear in from one to two months after the infected bark has been killed. They exhibit a wide variation in form and size but are of the typical *Gloeosporium* type. Arising from a mass of mycelium in the cortical tissue, they usually break through the epidermis of the bark. Occasionally they are concentrated in a small area and instead of breaking through, they lift the epidermis to form a blister. When the acervuli break through the epidermis, the stalk is almost non-existent, but when a bark blister is formed the stalks may be 1 to 2 mm. in length. There may be as many as 50 acervuli concentrated on a square centimetre of surface. The sporulating surface of the acervuli is generally round and convex, about .5 mm. in diameter, and fringed with a ray of mycelial strands. An active spore-bearing surface is whitish to tawny in color. Old acervuli are black. The stalk is grayish or black. The acervulus itself consists of a mass of parallel mycelial strands matted together, the central strands of which bear the conidiophores, which constitute the spore-bearing surface. The outer mycelial strands grow out to form a fringe-like ray around the convex centre. Acervuli vary widely in shape, due at times to the coalescing of neighboring acervuli, and at times, to conformity with the shape of cracks on the cortical tissue from which they arise.

The majority of the acervuli are produced on newly killed bark in from a month to six weeks after the death of the tissues. A few additional acervuli appear on this area, however, over a very considerable period of time. Some individual acervuli produce several crops of spores. The conidiophores after producing spores resume a mycelial growth. The stalk of the acervulus is elongated and new conidiophores are formed again on the ends of the elongated mycelial strands. Acervuli have been observed in which this process has been repeated eight or ten times. Through such means the fungus is able to provide continuously an abundant supply of viable spores throughout the entire season.

The ends of the mycelial strands in the centre of the stalk constitute the conidiophores. These are branched several times and on their ends the spores are borne acrogenously. The conidia are hyaline, usually non-septate until just before germination, 10 to 15 μ in length by 3 to 4 μ in diameter, somewhat variable in shape, with rounded ends and either straight or slightly curved. Commonly they contain two or more vacuoles. Spores germinate readily in 2% sugar solution and tests have shown that viable spores occur throughout the orchard during the entire year.

II. Woolly Aphis in its Relation to the Disease

As early as 1926, evidence showed a relation between annual canker enlargement and presence of aphides. Conclusive evidence was sought as to the exact relation of these two in the occurrence of the disease, and it is now considered that the aphis is the inoculating agent without which the fungus is unable to enter the host tissue and renew parasitic activity.

A technique for excluding aphides which at the same time would not adversely affect the development of the fungus in the host was developed by the wrapping of cankers with cotton cloth. All cankers wrapped were selected from those in which a spread of infection had occurred in the same spring in which wrapping was done. The cankers were not otherwise disturbed; the dead phloem was left in place and no wood tissue was removed. Wrapping was done in May before aphis infestation became general and a high percentage of cankers remained free of this insect throughout the season. Cankers have been so wrapped throughout a period of four years during which there occurred wet and dry seasons and severe and mild winters. In some 300 aphis-free cankers counted, not one case of canker infection occurred, although some of the wraps had been in place for three years. Of the latter cankers many had completely healed over and sealed.

On the other hand, in the case of wrapped cankers in which aphides developed, it was found that vigorous colonies usually resulted from the protection from predatory insects afforded by the wraps. Of 144 such cankers, all but 2 were found to show spread of canker infection.

The occurrence of spread of infection in practically every canker where aphides were present, and the 100% absence of spread in the cankers where no aphis attack took place, provide convincing evidence that the fungus is unable to accomplish successive infections in cankers without the presence of aphides.

A few cankers were also induced in a caged tree in a canker-free district by the transporting of diseased bark and aphides from an infected district. Colonies established themselves on pruning calluses and from two such areas the fungus was later isolated.

Experimental work was undertaken also to ascertain at what time of year aphides induced successful inoculation. By the use of paints and aphis repellents it was found that the exclusion of aphides during May, June and July

was of no great benefit, inasmuch as infections did not arise from insect activities during that period. From August to December, however, it was found necessary to keep aphides out, as infection resulted in a very high percentage of cases following insect attack during these months.

III. The Influence of Exposure to Low Temperatures

From observational evidence it has long been clear that there is a relation between winter temperatures and the occurrence of the disease, the amount of spread in the latter always being much greater after severe winters. From experimental evidence it is now clear that there is not only such a relation but that the disease is entirely incapable of advancing in the tissues of the tree without the action of freezing temperatures upon these tissues. Such action, however, is in no wise sufficiently severe to be in any way classed as producing what is commonly understood as winter injury.

An experiment to protect aphis-infested cankers from all exposure to frost was accomplished through the enclosing in November of the lower portion of a tree in a large box, about 8 ft. to a side and 8 ft. high, and filling the latter with sawdust. Artificial inoculations were also placed on the tree previous to filling, these being arranged so that some would be above the sawdust, and some well beneath the surface. A minimum thermometer was placed at a depth of a foot in the sawdust. In May after normal infection spread in the orchard was completed, the sawdust was removed. The minimum thermometer registered 36° F. In the cankers and the artificial inoculations below the six-inch depth, no single case of spread of infection in uninjured tissue occurred. All the cankers within the top six inches showed slight spread of infection. Of the cankers above the sawdust 98% showed spread, and of the inoculations 100%, the infection in the latter having run as much as 1½ to 2 in. longitudinally.

Therefore, though the fungus was present and the protected cankers were actually aphis-infested at the time of filling in the sawdust, the fungus was unable to advance in any single instance where freezing temperatures had not prevailed. This exposure to winter temperatures is therefore in itself a limiting factor in the occurrence of the disease.

The amount of exposure to winter temperatures determines largely the extent to which the infection penetrates the healthy tissue each season. It explains the occurrence of the more extensive infection in the northern than in the southern sections of the Okanagan Valley. During the season 1931-1932 for example, the average maximum spread in 89 aphis-infested cankers in the north was $\frac{7}{8}$ in. and in 82 cankers in the south, $\frac{1}{2}$ in. The average maximum spread from 142 inoculations in the north was 1 in. and in the south, $\frac{3}{4}$ in. Meteorological data show that in the north the prevailing daily minimum temperatures were lower than in the south.

The winters in the southern sections are not sufficiently mild, however, to afford an immunity to the general advance of the disease, as some workers believe. Where the disease is now established in these sections, it is advanc-

ing year by year and will eventually threaten the loss of many orchards. It is true that in these sections the disease is not yet as prevalent as in the north, but this condition is probably due to factors other than winter temperatures.

Winter Injury

There can be no doubt but that the conditions which will induce winter injury to the trees also greatly aid the fungus in its parasitic attack. It has often been observed that where trees are grown in situations where such injury is common, canker enlargement after severe winters is often very excessive. Under such conditions, trees have in one season been killed outright from a combination of these two factors. It should be remembered, however, that winter injury is not a necessary forerunner of normal or even extensive canker enlargement..

Precipitation

Perennial canker thrives in arid districts where other fungi such as scab, *Venturia inaequalis* (Cke.) Winter, and apple tree anthracnose *P. malicorticis* are unable to make any headway. The fact remains, however, that perennial canker is more prevalent in the northern sections of the Okanagan where precipitation is higher than in the southern.

It is suggested that the prevalence of the disease in these sections is due to the more common occurrence of the causal organism existing there as an ordinary saprophyte. In one such northerly section, the Salmon Arm district, where precipitation is relatively high, perennial canker does not occur and woolly aphis has not been present. Occasionally, however, there can be found on the dead bark of winter-injured trunks in this area, the fruiting bodies of a fungus similar in all respects to that of *G. perennans*. Spores of this fungus produce on artificial media a culture identical with the perennial canker fungus. Moreover, in a recent outbreak of woolly aphis in one orchard of this section there has occurred the characteristic killing back of the callus tissue on infested calluses. From these there was isolated this spring a fungus identical in culture to *G. perennans*. It seems highly probable, therefore, that the general occurrence of perennial canker throughout this section in the future will depend only on the spread of this new woolly aphis infestation. The fungus appears to be already there.

Precipitation is undoubtedly instrumental in inducing button cankers. These however rarely penetrate to the cambium and of themselves constitute no serious disease problem.

In the fruit the rot is also attributable in the main to the washing of spores to the ripe apple.

Relation of Pruning to the Disease

In pruning, wounds are made which allow of fungus establishment. Infection arising from this cause is not extensive and since such infections never enlarge of themselves and healing takes place after the initial infection is complete, no great danger arises from this source.

Variety, Cultural Practices and Vigor of the Tree

A wide range of susceptibility to the disease exists among the various varieties. The more susceptible varieties in this district are Spitzenberg, Yellow Newtown, Rome Beauty and Jonathan. The more resistant varieties are McIntosh, Delicious, Winesap and Wealthy. In an individual variety, little variation in susceptibility exists in any given district under the normal range of cultural conditions. Individual trees are but seldom found that show a distinctive resistance for that variety.

Trees, however, when grown under extremely unfavorable environmental conditions will exhibit a much stronger susceptibility than is generally characteristic of that variety. One orchard under observation growing in a "frost pocket" and of a variety generally considered only slightly susceptible, displayed a remarkable development of susceptibility, the trunk and main branches of the trees becoming girdled by cankers and death ensuing in one year. Non-susceptible varieties growing under similar conditions have been observed to develop extensive cankers typical of those on a quite susceptible variety.

Excessive advance of the fungus occurs also when the disease has progressed to a stage where cankers are large and numerous on the trunk and main branches. The interference with the food supply and the resultant general devitalization of the tree may give rise to an extraordinarily rapid advance of the fungus with frequent coalescing of cankers and girdling of large limbs. Great and sudden losses frequently result in such situations and these may be considerably aggravated by winters of extreme severity.

General Considerations for Control

Satisfactory control measures for the disease have not been developed. The abundance and continued production of crops of spores over long periods render a spray program of little benefit in keeping the surface of the host protected. The persistence of woolly aphides against insecticides and their capability of rapid reproduction makes their elimination difficult and expensive.

Individual cankers may be treated with some success by painting with aphid repellents. Rape seed oil and castor oil have shown some value when applied undiluted. Before painting the canker all dead bark should be removed. Where cankers are numerous, however, this procedure also is difficult.

Canker development may be delayed by keeping up the vitality of the tree and so inducing faster callus formation. Though fungus advance appears not to vary greatly in fast and slow growing trees, by inducing a vigorous callus formation the tree is more nearly able in its healing processes to keep pace with the advance of canker enlargement.

Pruning in diseased orchards may be delayed until late winter or early spring. The results of inoculations indicate that infection may be readily induced by wounding during November and December, but not if wounding is done later.

Discussion

Among the various investigators who have worked on this problem, there exists considerable uncertainty as to the influence which various factors have on the occurrence and severity of the disease. The purpose of this paper has been to present results of further investigations and through the co-ordination of this work with the author's former findings, to present an interpretation of the perennial canker problem.

The results obtained by Zeller and Childs, Cooley and Miller and the writer agree in indicating that the fungus *G. perennans* is the agent through the activity of which the actual killing of the tissues occurs in the production of the disease. In the papers published to date, however, there has been no presentation of the complete life history of the fungus and there exists a vagueness as to the specific conditions governing its pathogenicity. The evidence presented herein stresses the importance of the saprophytic phase of the fungus as being sufficiently developed to ensure its existence quite independent of any parasitic action. As a parasite it has been found to be strictly annual in the tissues of the tree. Its parasitic phase in the host tissues is definitely limited to one season's development. When this is complete, it cannot again of itself cause a new infection from the old. Moreover, the evidence seems clear that no new infection can arise in the canker through the germination of spores on the surface of the callus. For the formation of cankers therefore there must occur a series of successive inoculations into the callus tissue. It is evident that the fungus cannot in itself produce a typical perennial canker.

Since the unaided fungus is not sufficiently parasitic to bring about successive annual enlargements in the canker, it must rely on some outside agency to assure its inoculation each year into the host. These experiments indicate clearly that in the production of cankers it is the woolly aphis which, through its feeding activities on the callus, assures these successive inoculations. It is true that inoculations or infections may arise occasionally entirely without the aid of aphides, such as infections that follow pruning operations or the occurrence of button cankers. In such cases, however, the infection is entirely annual in nature and successive enlargements from these infections do not occur. Consequently, a typical canker is never produced.

In the experiment on the effect of excluding aphides from cankers which was carried on over a period of years on two varieties and in two different districts, and which involved treatment of over 400 cankers, no case of new infection was found to occur in any canker from which aphides were excluded, and in those in which aphides were allowed to remain, new infection did occur in every canker except two. There can be no doubt that the production

of perennial canker in trees is entirely dependent upon a successive series of autumn inoculations in the callus tissue through the agency of the woolly aphids.

Successful inoculations are not effected at all seasons of the year by woolly aphides. Experimental evidence has shown that aphides, feeding on calluses during the late spring and early summer, do not bring about the occurrence of infection. Only when infestation with the insect occurs during the late summer and fall is there effected an inoculation which culminates in the production of infection.

In conjunction with the inoculation by aphides of the host tissue in the fall, there appears to be a third factor necessary in the occurrence of the disease. This factor is the bringing of the host tissue into a physiological condition suitable for the invasion of the fungus. In the creation of cankers, the enlargement occurs in the late winter and early spring. It is quite apparent that the extent of this enlargement is largely dependent on the severity of the winter. During winters characterized by long periods of low temperatures, canker enlargement is always excessive. After mild winters, on the other hand, it is always slight. The results of experiments show that where cankers are not exposed to any frost during the dormant season, no enlargement takes place, although all the other prerequisites for successful inoculation have been provided. Exposure to winter temperatures is, therefore, a necessary concomitant in perennial canker.

Various other factors, such as precipitation and cultural conditions, are associated with the occurrence and severity of the disease. Their influence, however, is secondary and they are not essential in the development of the canker.

Satisfactory control measures from an economic standpoint have not been developed. There are three avenues of approach. First, by removing dead bark from the cankers the sources of spore contact with aphides are withdrawn. Second, the trees may be kept in vigorous growing condition with some benefit, inasmuch as vigorous callus growth tends to close-in the canker and more nearly keep pace with the annual advance of infection in the previous year's callus. The progress of the canker in encircling the limb and causing devitalization is thus somewhat retarded. Third, the elimination of woolly aphides would make possible the eradication of the disease in the orchard. The cost, however, of effectively controlling aphides in accordance with present recommendations is prohibitive from an economic standpoint. Aphides may be excluded from individual cankers through the use of insecticidal paints or repellents. But here again, where the disease has become established and cankers are very numerous, the task is both burdensome and expensive.

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FIRE HAZARDS IN THE USE OF OXIDIZING AGENTS AS HERBICIDES¹

By W. H. COOK²

Abstract

The combustibility of organic matter-sodium chlorate mixtures in various proportions has been tested at different relative humidities. It was found that mixtures containing more than 10% of sodium chlorate are sufficiently combustible to be hazardous at all relative humidities below 75% but are non-hazardous at higher humidities. Data were obtained showing the minimum moisture content required for protecting such mixtures and this information may be used to calculate the approximate amount of any water-absorbing chemical that has to be added to sodium chlorate to render it safe. Admixture of calcium chloride or magnesium chloride with the sodium chlorate renders the mixture safe when these protecting salts form one-half and one-third respectively of the resulting herbicide. Judging from the chlorate content the above sodium chlorate-magnesium chloride mixture would be the most effective "safe" herbicide of those tested, but it would be about only half as toxic as pure sodium chlorate. Several other salts were tested as protecting agents but they were either ineffective or resulted in the decomposition of the chlorate. Combustibility tests were also conducted on barium chlorate- and calcium chlorate-organic matter mixtures. These mixtures are less inflammable than those containing sodium chlorate but are sufficiently combustible to be a fire hazard in districts where the atmospheric humidity is low. Addition of one part of anhydrous calcium chloride or magnesium chloride to two parts of barium chlorate results in a safe mixture. Sodium dichromate-organic matter mixtures are much less combustible than those containing chlorate, but these mixtures also are somewhat dangerous at ordinary humidities when there is a high proportion of sodium dichromate in the mixture. When 10% of anhydrous calcium or magnesium chloride is added to the sodium dichromate the resultant mixture is safe under ordinary atmospheric humidities, regardless of the proportion of organic matter present. Mixtures of sodium chlorate and sodium dichromate with organic material are extremely combustible.

Introduction

The fire hazards associated with the use of strong oxidizing agents as herbicides have been made the subject of this investigation. Sodium chlorate is an example of the class of chemical concerned and is the one most commonly used for killing perennial weeds. This compound is relatively safe to

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handle in the pure form but under farm conditions it commonly becomes mixed with organic material, and the resulting mixture is readily ignited by a spark, heat or friction. Solutions of sodium chlorate can be handled quite safely, but unfortunately they dry rapidly and completely, leaving contaminated wooden vessels, clothes, or plant material an extreme fire hazard. A possible method of reducing this risk is to mix sodium chlorate with chemicals of high water-absorbing capacity, or with other non-oxidizing chemicals of good herbicidal power, the latter acting as a diluent and a fire preventive when in contact with organic material. Such mixtures have been investigated, particularly in regard to the relative humidity and the proportions of the various chemicals necessary to reduce the danger of fire. The alternative possibility of supplying the chlorate ion in another combination, such as calcium chlorate and barium chlorate has also been studied from the standpoint of fire risk.

Sodium dichromate is also used to some extent as a herbicide. Preliminary work (2) indicates that this compound is quite toxic to annual weeds, but its value for killing perennials is still doubtful.

Review

Sodium and potassium chlorate form a major part of the so-called "chloratites" and "cheddites" which are explosives of the "blasting" type. A typical cheddite contains 70 to 90% potassium chlorate mixed with an aromatic nitro compound and paraffin. The data given in the International Critical Tables (9) show that these explosives are more sensitive to shock, judging from the fall-hammer test, and have a higher heat of explosion, than black powder. The shock sensitivity and heat of explosion will naturally depend on the amount, kind, and physical state of the combustible material, but the above comparison gives some idea of the kind of explosive resulting when sodium chlorate is mixed with organic material.

The fire risk involved in the use of sodium chlorate has been recognized by those who have recommended its use as a herbicide. Nearly every circular and bulletin dealing with the herbicidal qualities of sodium chlorate includes warnings about its dangerous character when mixed with organic material. An editorial in *Industrial and Engineering Chemistry* (5) issues a warning about the use of sodium chlorate as a farm herbicide. This article states that sodium chlorate packed in metal containers is safe as long as it remains in the closed container, and of itself is not dangerous, inflammable, nor explosive. It is pointed out however that this safe chemical becomes an extremely dangerous one when the container is opened on the farm where admixture with organic dusts will result unless every precaution is taken.

In spite of the above warnings some bad burning accidents have occurred from the use of sodium chlorate. The writer of the above editorial claims to have been informed that fields sprayed with chlorate have ignited spontaneously on hot days; that vehicles used for carrying spray solution have later taken fire, probably owing to a jar or the heat of the sun; and that buildings

have been destroyed by fire apparently as the result of the solution drying or of dust settling on the chlorate. Deem (3) reports that a man's clothing, which had become wetted with chlorate solution, caught fire several days afterwards, in spite of the fact that he had soaked the clothing overnight in a tin of water after the spraying operation. The writer knows of two instances in which clothing, soaked with chlorate, has been accidentally ignited by friction after drying.

It is difficult for the non-technical man to realize the extremely dangerous character of sodium chlorate. Knowing nothing about the nature of the compound, he does not realize that although the pure chemical and its solutions are non-combustible, a mixture of it with organic matter yields material even more dangerous than gasoline, since it can be ignited by friction and shock as well as by a spark. Moreover, even if sodium chlorate is handled cautiously, it is almost certain that some of the chemical will come in contact with wagons and other farm equipment, unless special machinery is used for handling it, an arrangement which is impracticable on the ordinary farm. Indeed the sprayed plants themselves constitute fire hazards. To overcome this risk Loomis, Bissey, and Smith (7) have recommended that the top growth of the plants be removed before treating, and the chlorate salt applied to the soil. They claim that this method is as effective as the application of the spray to the growing plants.

Considering all factors it would seem best, in the interests of safety, to mix the chlorates with other chemicals which would reduce the fire hazard to a minimum, and if possible to have the resulting mixture possess, as nearly as possible, the same herbicidal power as the original material.

The possibility of reducing the fire hazard by the addition of other chemicals has been recognized by the manufacturers of commercial chlorate herbicides. Analyses of several chlorate weed killers show that approximately 60% of the dry solid is sodium chlorate and 40% is calcium chloride. Calcium chloride is much less toxic to plants than sodium chlorate and may be regarded as practically inert material. The chief function of the calcium salt is to reduce the fire hazard by absorbing moisture, although it is also claimed to improve the herbicide by preventing the chlorate from drying on the leaves and then blowing off. Arny, Bridgford, and Dunham (1) state that the presence of calcium chloride in Atlacide, a commercial herbicide, reduces the fire hazard, but does not remove it entirely.

Other commercial weed killers contain on a dry basis from 50 to 90% of sodium chlorate and from 10 to 50% of sodium carbonate. The reason for the admixture of the carbonates is not stated by the manufacturers, but it could scarcely be expected to reduce the fire risk, at ordinary atmospheric humidities, by the retention of moisture. At the temperature of combustion the decomposition of the carbonate with the evolution of carbon dioxide might retard the spreading of flame. Sodium carbonate might also reduce the fire risk by acting as a diluent, but this would also tend to reduce the herbicidal power of the mixture proportionally, since sodium carbonate alone

is less toxic to plants than the chlorates (2). It is possible, on the other hand, that the alkaline reaction which the carbonate would impart to the solution might increase the herbicidal power of the chlorate somewhat, but as yet there is little reliable information on this point.

Brief mention should be made of the explosive properties attributed by some writers to pure sodium chlorate, for even in the absence of organic matter this compound may break down into sodium chloride and free oxygen. Opinion seems to differ on this point, some authors (1, 5) believing that the pure chemical is quite safe while others (8, 12) consider it dangerous. Probably none of these investigators have studied the reaction critically. Lewis and Randall (6) from a consideration of the free energy and heat of formation of potassium chlorate, state that this chemical by itself is unstable, but would hardly be violently explosive. Since this statement will also apply to sodium chlorate, there seems to be little danger of this compound exploding. Mixing chlorates with water-absorbing chemicals in order to reduce the fire hazard when it is in contact with organic material would doubtless render the pure sodium chlorate entirely safe.

Experimental Material

The organic materials with which chlorates may come in contact will vary considerably in their inflammability. It was therefore necessary to select a readily combustible material for test purposes, in order that the protective action of any particular chemical or treatment might apply generally. Whatman No. 50 hardened filter paper was chosen first since it is essentially pure cellulose and therefore more completely combustible than the organic materials which will usually be met with in practice. Even this hardened paper, however, disintegrated somewhat on being soaked in the various solutions, and although it was used in certain experiments, it was found that cloth was more convenient to handle and yielded more accurate data. The cloth used was a 12-oz. duck. This material was thoroughly washed and rinsed with distilled water before use. It contained about 0.5% ash on an oven-dry basis.

Experimental Method

The method employed consisted essentially of treating the organic material, either cloth or filter paper, by soaking it in a solution of the oxidizing agent under test, then conditioning the treated material at a known relative humidity, and finally testing the inflammability of the treated and conditioned material.

Before starting a series of tests the cloth to be used was cut into two-inch squares, weighing about $1\frac{1}{4}$ gm. each. These pieces were numbered and a piece of cotton thread attached to each for hanging in the conditioning chamber. When filter paper was used three pieces 7 cm. in diameter had to be fastened together to get about the same weight of material. A piece of copper wire of known weight was used to fasten the pieces together and to provide a hanger.

The first step was to obtain the dry weight of the test material. A large number of pieces were placed in the conditioning chamber over sulphuric acid, and allowed to remain until their weights reached equilibrium. A few of these pieces were then placed over phosphorus pentoxide *in vacuo* at room temperature and if a further loss in weight took place the weights of the pieces in the conditioner were calculated to a bone-dry basis. This drying procedure was adopted since it was also suitable for treated material that could not be oven-dried.

The test pieces were treated by soaking them in solutions of the oxidizing agents to be tested. The solution under test was placed in a vapor-tight vessel and the pieces submerged for one hour. The material was then raised up out of the solution and allowed to drain at the vapor pressure of the solution for two hours in the same tight vessel. After draining, the pieces were allowed to dry in the room before placing them over sulphuric acid in the conditioning chamber. When the test pieces had reached equilibrium over the sulphuric acid, a few pieces were dried over phosphorus pentoxide as previously described for the untreated material. The amount of oxidizing agent, taken up from the solution, could then be calculated from the dry weights of the untreated and treated material.

By treating the test material with solutions of the oxidizing agent, as described above, a uniform treatment of all material was ensured. Furthermore the percentage of oxidizing agent in the treated material could easily be varied by using solutions of different concentrations, and any proportion or concentration of other salts, to be tested for protective action, could be easily obtained by varying the composition of the solution.

In order to determine the combustibility of the treated material under different atmospheric conditions it was necessary to conduct tests over a wide range of relative humidities. It was also important to determine the minimum moisture content required to give protection to the treated material, for these results could be used to estimate the amount of any water-absorbing chemical that would have to be added to the oxidizing agent to remove the fire risk. For these determinations it was necessary to construct a conditioning cabinet capable of maintaining the required relative humidities, and fitted with a balance so that the individual pieces could be weighed.

A drawing of the conditioning chamber is shown in Fig. 1. It consists of a closed chamber having a removable lid which fits into an oil seal. A window and small door were provided in the front of the chamber. This door, which was used for removing the individual pieces for test, consisted of an outer metal door, and an inner one constructed from four overlapping pieces of dental rubber dam stretched over a metal frame. By removing the outer door it was possible for the operator to put his hand into the chamber and take out the test pieces with the minimum exchange of air since the rubber passed quite snugly over the contour of the hand and wrist. The window and outer door were sealed in with plasticine. The chamber was provided with a De Khotinsky thermoregulator, two carbon filament lamps for heating, a

copper water-cooling coil, an air circulating fan, a turntable for holding the test pieces, and a rack for three glass trays. The temperature was kept at $25^{\circ}\text{C.} \pm 0.1^{\circ}\text{C.}$ throughout these experiments. The relative humidity was controlled by placing concentrated sulphuric acid or a series of saturated salt solutions having known vapor pressures (11) in the three trays. The humidity actually attained was determined by drawing a measured volume of the conditioned air through two absorption tubes containing sulphuric acid and pumice. The volume of air was measured by a standardized Boys gas meter and the necessary corrections made for temperature, etc.

The turntable consisted of two metal plates fastened to a vertical shaft, the upper plate carrying two rows of hooks, at different levels, and the lower plate one row. This unit was manipulated from outside the cabinet and it could be moved up and down as well as rotated, so that any hook could be brought opposite the balance hanger. A small manipulator, similar to the rider carrier on a balance, fastened to the side of the cabinet, was used to transfer the pieces from the turntable to the balance and *vice versa*. With this equipment over 90 test pieces could be conditioned at one time. •

The test pieces were allowed to remain in the chamber until they had reached equilibrium as determined by weighing. In order to weigh the pieces without removing them from the humidostat, a Jolly balance of the Cenco Precision type was employed. From the bottom of the balance spring a straight piece of aluminium wire with a small hook at the lower end extended down into the cabinet through a small hole in the lid. This hole was stoppered when the balance was not in use. The spring extension of the balance is measured by increasing the length of the telescoping stand, and reading the increase on a vernier scale. By using a light steel spring, protected from air currents by a celluloid guard, it was possible to weigh the pieces to the nearest two milligrams.

As explained previously, the dry weight of the treated pieces, over sulphuric acid, was obtained first. The sulphuric acid in the glass trays was then removed and replaced with a saturated solution of lithium chloride (relative humidity 15% (11); actually 11.5 in these tests). All of the test pieces were then reweighed and some of them tested for inflammability when they reached equilibrium. Other saturated salt solutions of progressively increasing vapor pressures were used in turn until the test pieces had attained a moisture content at which they would no longer burn. By this procedure the dry weight of the pieces, the weight of the chlorate absorbed, and the moisture content at each relative humidity was obtained. The large number of test pieces weighed at the low relative humidities gave reliable averages for the moisture content where the weight increases were small. At high relative humidities, when fewer test pieces remained, the weight increases were correspondingly greater and fewer pieces were necessary to attain the same relative precision.

In developing an apparatus for testing inflammability it was found that cloth and paper, treated with sodium chlorate, and dried, could be ignited by dropping a weight on the material. This modified fall-hammer test

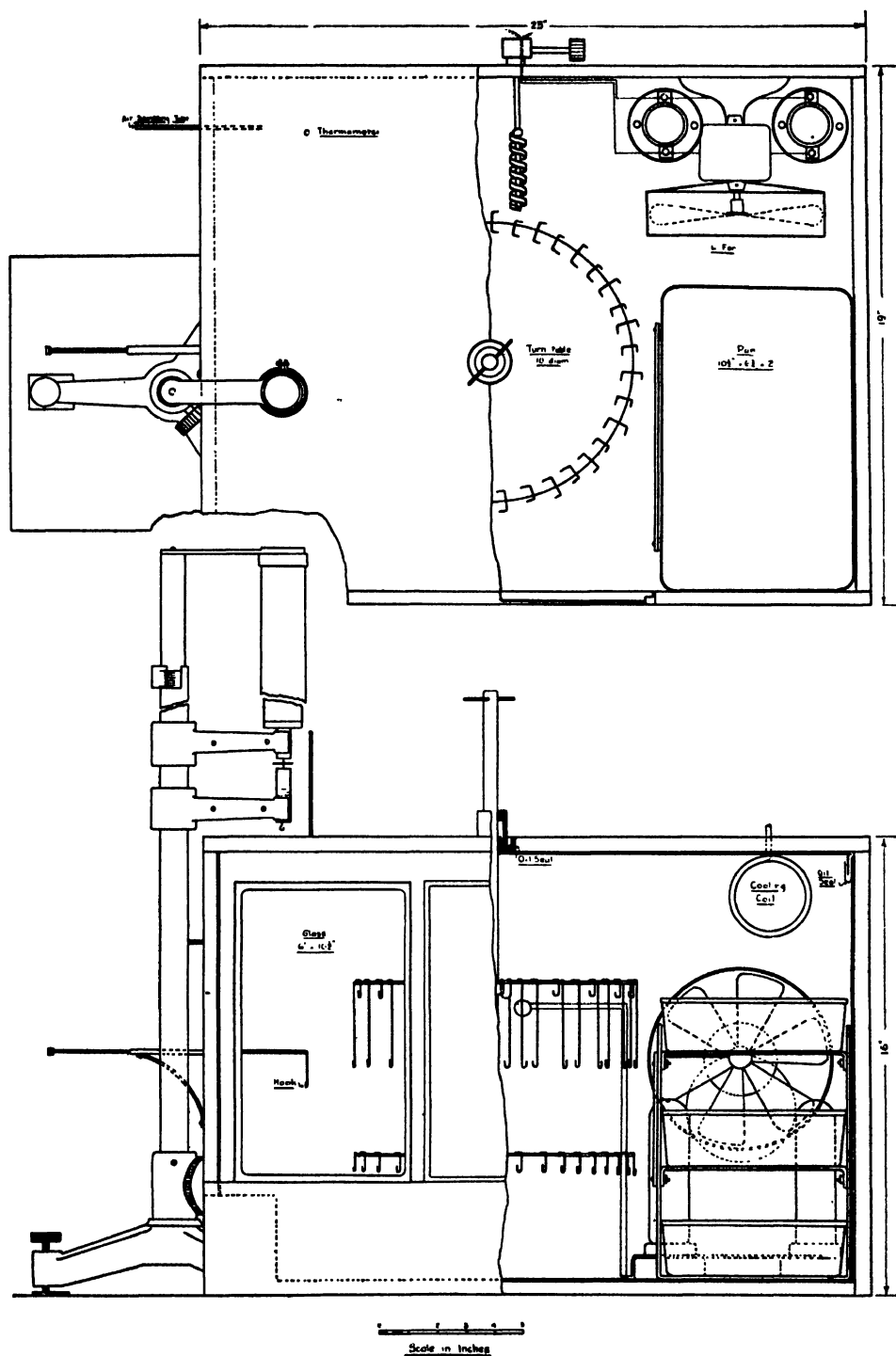


FIG. 1. Humidostat for conditioning test materials.

would have required quite elaborate apparatus, however, to test the fire hazard of samples conditioned at different relative humidities. Considerable time is required to establish the minimum height the hammer must fall to explode the material, and during this time the test piece would have changed in moisture content if the apparatus were set up in an ordinary room. Construction of a humidostat for this apparatus would have been possible, but as each combustion would have altered the temperature and humidity conditions temporarily the carrying out of an extensive series of tests would have been time-consuming in the extreme. It was consequently considered desirable to devise a test that could be conducted so rapidly that the material would not change significantly in moisture content while being tested under ordinary laboratory conditions.

The apparatus finally designed for this purpose was essentially an induction coil and spark gap.

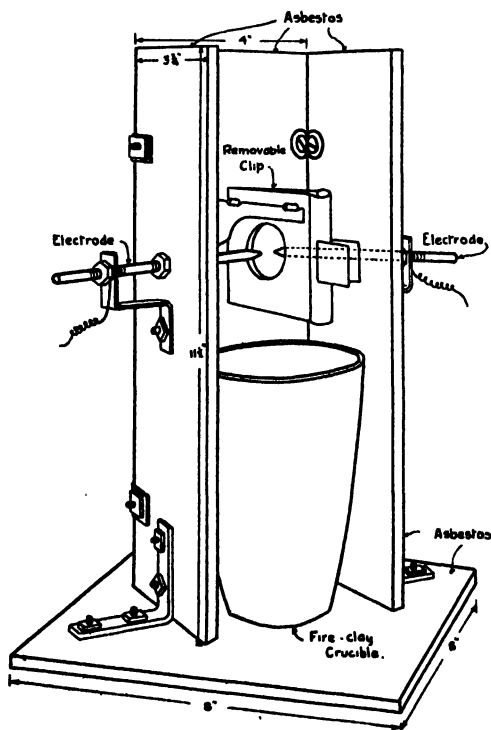
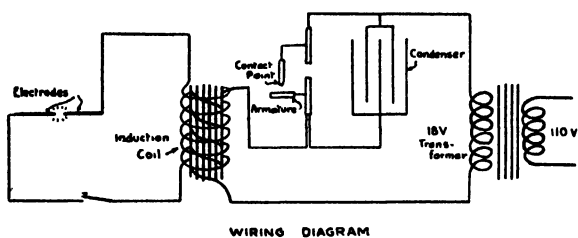


FIG. 2. Apparatus for testing combustibility.

The test piece, held in a suitable clip, was placed between the electrodes, and the spark permitted to pass through the material for a fixed period. The electrodes were held about 1 cm. apart, and the current used was sufficient to burn a hole 2 to 3 mm. in diameter, in untreated cloth, in five seconds. A drawing of the complete apparatus which consisted of a 110- to 18-volt step-down transformer, an induction coil, a switch, and a spark gap enclosed in a combustion chamber, is shown in Fig. 2.

A criterion of what constituted a fire hazard had now to be established. In untreated material, regardless of the relative humidity at which it was conditioned, the spark usually burnt a hole 1 to 2 mm. in diameter in about two seconds. This hole would increase in diameter as the spark continued and after five seconds the material would usually be ignited and

would then burn slowly with a free flame. Obviously the small area traversed by the spark was no longer at the moisture content at which it left the conditioning chamber so that any criterion of inflammability had to be taken from the rate of spread in the surrounding material. The inflammability of the treated material was finally classified into three groups as follows:—

1. *Non-hazardous*.—No spontaneous spread of flame when subjected to a spark of five seconds' duration, the burnt hole being of similar size, or smaller, than that burnt in untreated material by a five-second spark.

2. *Doubtful hazard*.—Hole burnt by a five-second spark larger than that obtained in untreated material, with occasional spontaneous spread in a few of the pieces.

3. *Hazardous*.—Material in which flame spread spontaneously after ignition by a spark of less than five seconds' duration.

Plate 1 shows the holes burnt in pieces of cloth and filter paper which have been (a) untreated; (b) treated with an oxidizing agent and are considered a doubtful hazard, since spontaneous spread is quite common in test pieces having a hole of this size from a five-second spark; and (c) treated with an oxidizing agent plus a protecting agent and are considered safe. Obviously hazardous material could not be photographed as it was completely burnt.

Experiments with Sodium Chlorate

In the first experiment, test pieces, cut from cloth and filter paper, were soaked in 10, 20, 30, 40 and 50% sodium chlorate solutions, dried over sulphuric acid, and then conditioned at 25° C. at the relative humidity existing over the various saturated solutions listed in Table I. This table gives two values for the relative humidity; that given in the International Critical Tables (11) and the average values as actually determined in these experiments. These agree quite closely in most cases, but where they do not agree the experimentally determined values are believed to represent the conditions existing in these experiments. The relative humidity maintained by the various solutions at 25° C. is not always given in the International Critical Tables, so the temperature to which the listed values are appropriate is also given in Table I.

TABLE I
RELATIVE HUMIDITY OVER VARIOUS SATURATED SALT SOLUTIONS

Salt	Inter. crit. tables		Experimental	
	Temp. ° C.	R.H. %	Temp. ° C.	R.H. %
LiCl . H ₂ O	20	15	25	11.5
CaCl ₂ . 6H ₂ O	24.5	31	25	29
K ₂ CO ₃ . 2H ₂ O	24.5	43	25	43
Na ₂ Cr ₂ O ₇ . 2H ₂ O	20	52	25	53
NH ₄ NO ₃	25 (approx.)	62 (approx.)	25	61
NH ₄ Cl and KNO ₃	25	71.2	25	65.5
(NH ₄) ₂ SO ₄	25	81.1	25	78

KEY TO PLATE I

1. Holes burnt in untreated cloth by a spark of five seconds' duration.
2. Holes burnt in untreated filter paper by a spark of five seconds' duration.

Note small holes and large darkened area due to atmospheric oxidation.

3. Holes burnt in cloth soaked in sodium chlorate and conditioned at a relative humidity at which it is a doubtful hazard. Spark of five seconds' duration.
4. Holes burnt in filter paper soaked in sodium chlorate and conditioned at a relative humidity at which it is a doubtful hazard. Spark of five seconds' duration.

Note larger holes with almost complete absence of darkening from atmospheric oxidation.

5. Holes burnt in cloth soaked in sodium chlorate and conditioned at a relative humidity at which it is not a fire hazard. Spark of five seconds' duration.
6. Holes burnt in filter paper soaked in sodium chlorate and conditioned at a relative humidity at which it is not a fire hazard. Spark of five seconds' duration.

7. As in 3, but soaked in sodium dichromate.
8. As in 4, but soaked in sodium dichromate.

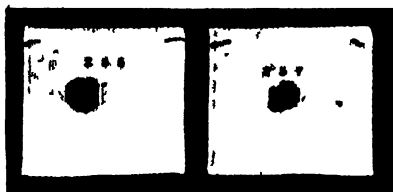
9. As in 5, but soaked in sodium dichromate.
10. As in 6, but soaked in sodium dichromate.



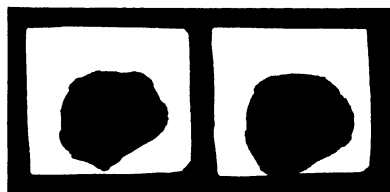
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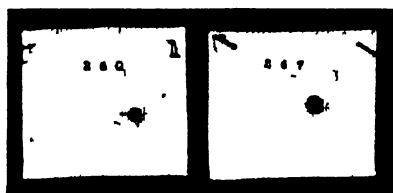
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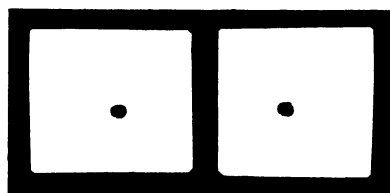
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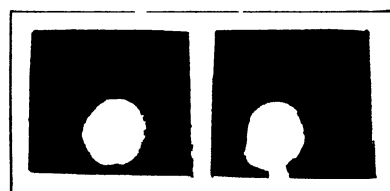
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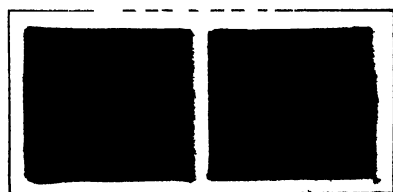
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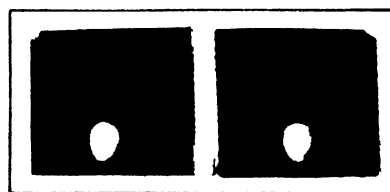
7



8



9



10

Showing test pieces of varying degrees of combustibility.

The amount of sodium chlorate taken up by the test pieces varied from about 13% to about 82% of the dry weight of the cloth or filter paper. Fig. 3 A shows that there is an almost linear relation between the percentage of sodium chlorate taken up by the test pieces, and the concentration of the solution in which they were soaked. In every case the filter paper held more salt than the cloth.

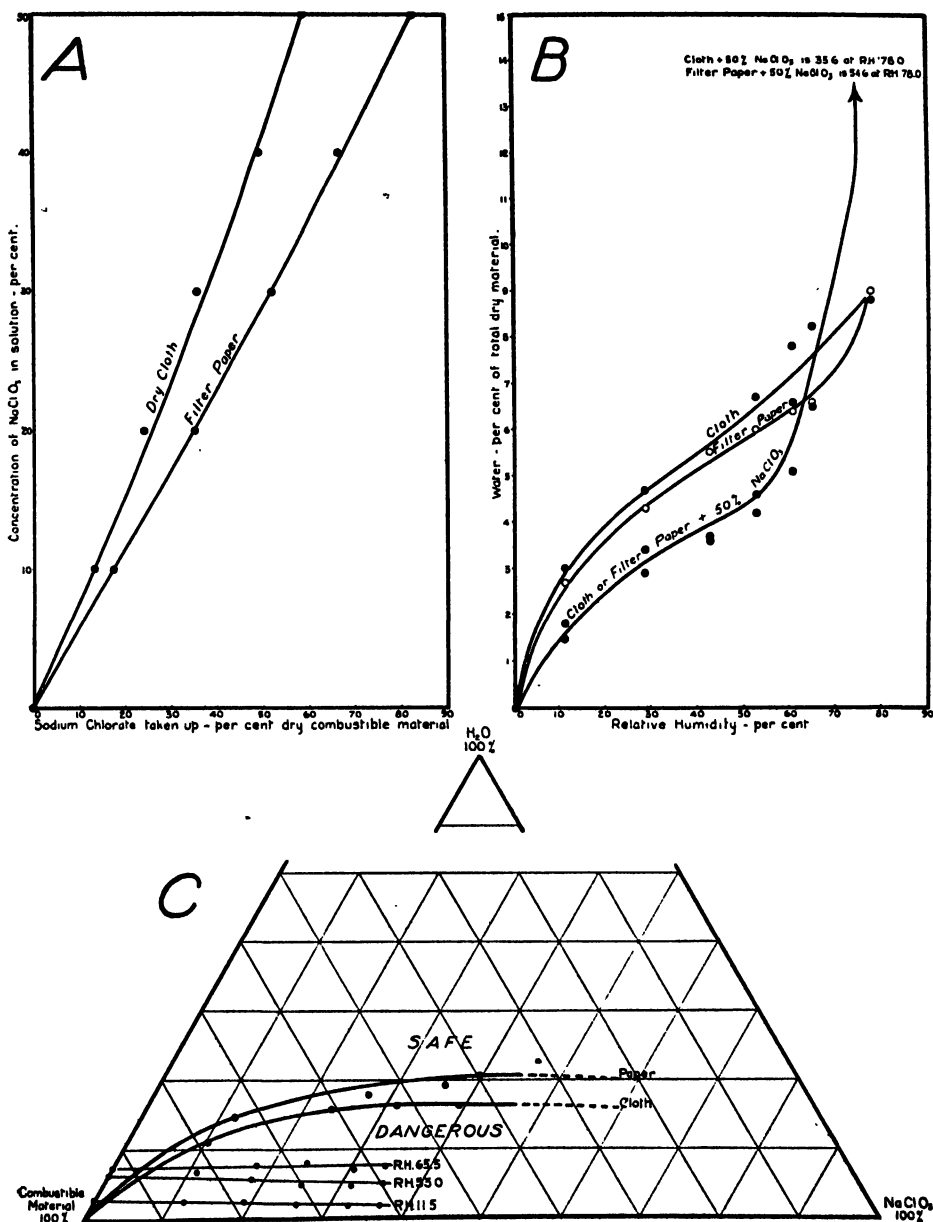


FIG. 3. Properties of test materials treated with sodium chlorate solutions.

Fig. 3 B shows the amount of moisture taken up by untreated cloth and filter paper, and also the moisture taken up by the same material after treatment in 50% sodium chlorate, when exposed to atmospheres of different relative humidity. In this figure the moisture content is expressed as a percentage of the weight of the total dry material, regardless of whether it was cloth only, or cloth and sodium chlorate. It is evident from the curves, that sodium chlorate does not take up moisture as readily as cloth, the curve for the treated pieces being below that of the untreated. This applies to all relative humidities below 75%. At this point the vapor pressure of the atmosphere is equal to the vapor pressure of a saturated solution of sodium chlorate (11) and higher relative humidities result in enormous increases in the moisture content of the treated material.

It was found that the test pieces soaked in any of the solutions of sodium chlorate were hazardous at any relative humidity below 75%. Material soaked in a 10% solution seemed to be a little less combustible than that soaked in more concentrated solutions. As would be expected, the filter paper test pieces burned more readily than the cloth. At relative humidities above 75% none of the test pieces would burn at all, regardless of the concentration of the solution in which the pieces had been soaked, and the class of combustible material. The reason for this is evident, since at relative humidities below 75% the moisture content of the material was less than 10%, while test pieces allowed to come to equilibrium at higher humidities contained over 35% moisture.

Although these results show the influence of relative humidity on the combustibility of sodium chlorate-organic matter mixtures, they give no information as to the minimum moisture content required to protect the material. Thus the moisture content of material treated with sodium chlorate increased sharply from about 10% at relative humidities below 75%, to over 35% at humidities just above 75%. The minimum moisture content required for protection must therefore be between 10% and 35%. It is important to know this value accurately, since it would enable the amount of calcium chloride or other water-absorbing substance of known water-holding capacity, required for protection, to be calculated.

In order to obtain this information a number of test pieces were prepared as before and placed in a saturated atmosphere. These pieces were not allowed to come to equilibrium, as in the previous experiment. Instead, one or two of the pieces were weighed at frequent intervals to determine their moisture content, and then removed for the combustibility test. This was continued until the material had taken up sufficient moisture to remove the fire hazard.

The results showed that the amount of moisture required to protect the test pieces varied somewhat with the proportions of the combustible material and of sodium chlorate they contained. In order to represent these results graphically a triangular diagram, shown in Fig. 3 C, has been employed. To use this type of chart it is necessary to express the amount of each com-

ponent as a percentage of the whole, so that the sum of the percentages is equal to one hundred. The triangle is equilateral, the distance from the centre of one side to the opposite corner representing 100. The corners of the triangle represent the three individual components, the sides represent mixtures of two components, and since every point within the triangle has the property that the sum of the perpendiculars from this point to the sides is equal to 100, it is evident that the composition of a three-compound system can be represented by fixing a point within the triangle. The fractional amount of a given component is then represented by the perpendicular distance of the point from the side of the triangle opposite the corner representing this component.

The upper curve in Fig. 3 C divides the filter paper-sodium chlorate mixtures into those which contain sufficient moisture to be safe, and those which do not. Points in the area above this curve represent systems that are safe while points in the area below the curve represent systems that are dangerous. The lower curve gives the same information for cloth, a less combustible substance. Tests made with leaves that had been treated with sodium chlorate indicated that they have a combustibility intermediate between the curves obtained with cloth and filter paper, but it was impossible to get reliable data with such material.

In order to extend the curves over as wide a range as possible, a few pieces of the test materials were soaked in a saturated sodium chlorate solution. This resulted in the dry material containing as high as 52% sodium chlorate. Higher percentages of salt would not adhere to the cloth when dry. The entire range of practical importance has been covered, however, and the curve has been extrapolated, as a dotted line, a short distance beyond the experimental points.

The straight lines in the lower left hand corner of the Fig. 3 C, represent the amount of water taken up by the different systems at relative humidities below 75%. No data are shown for the relative humidities above 75% as the material containing sodium chlorate took up so much moisture that some of the resulting solution drained off the pieces, making the weights unreliable. It is evident, however, that at all ordinary atmospheric humidities there is not sufficient water retained by sodium chlorate-organic matter mixtures, to provide protection.

The curves shown in Fig. 3 C enable the approximate amount of any water-absorbing chemical that has to be added to sodium chlorate to remove the fire risk when the latter is mixed with organic material, to be estimated. Examination of the curves also reveals that the amount of moisture-retaining chemical required for protection will depend on the relative amount of combustible material present. In practice the amount of herbicide that may come in contact with organic material is wholly uncontrollable. The only factor that can be controlled is the composition of the herbicide. The latter must therefore contain enough water-absorbing chemical to provide protection regardless of the amount of organic matter present.

The foregoing curves show that when organic matter becomes contaminated with a herbicide composed of sodium chlorate and a water-absorbing chemical in fixed proportions, the fire risk tends to decrease rather than increase if more herbicide is added. The reason for this is that each successive increment of sodium chlorate added to the organic material requires a smaller increment of water for protection.

For example, consider a mixed herbicide composed of two-thirds sodium chlorate and one-third anhydrous calcium chloride. At relative humidities as low as 22% this amount of calcium chloride will retain sufficient moisture to bring the water content of the mixture, on a wet basis, up to about 25%. When organic matter containing 5% moisture (Fig. 3 B) is added to this herbicide the percentage moisture in the resultant mixture will be some intermediate value. According to Fig. 3 C this mixture will not become dangerous unless it has a moisture content less than 20%, as this moisture content protects organic matter-sodium chlorate mixtures of almost any proportion. This moisture content will be reached when about one-quarter of the mixture is organic material. Further additions of organic matter will cause further reduction in the percentage moisture and the mixture will become dangerous. The diluting effect of the non-combustible calcium chloride, apart from its moisture-retaining power, will tend further to reduce the inflammability, but the extent to which this is effective can be determined only by direct experiment. The above reasoning shows, however, that the inflammability increases rather than decreases, within certain limits, as the quantity of the mixed herbicide in the mixture decreases.

Experiments with Sodium Chlorate Plus Protecting Agents

In selecting a salt for admixture with sodium chlorate in order to reduce the fire risk, the choice lies among chemicals which retain moisture and those which reduce the inflammability in other ways. The first type includes such chemicals as calcium chloride, and their efficiency will depend on the amount of water held, the form in which it is held, the vapor pressure of this hydrate, the vapor pressure of a saturated solution of the salt, and the atmospheric humidity to which any contaminated material may be exposed.

The second type will include ammonium phosphate and other chemicals which have been used for fireproofing wood, cloth, etc. The efficiency of these chemicals in reducing the combustibility of organic material will depend on their form of deposition on, and their penetration into, the material, more than upon their deliquescent properties. The special treatments commonly used in applying these materials are obviously impossible with herbicides. Soluble carbonates which decompose at the combustion temperature, with the evolution of carbon dioxide, might also be effective in reducing the fire hazard by checking the spread of flame.

Any non-combustible material mixed with sodium chlorate will reduce the inflammability of contaminated material by dilution regardless of its other properties. The effectiveness of such a substance will depend on the

amount present. Obviously salts of high herbicidal power can economically form a much larger proportion of the herbicide than those that are merely inert material from the toxicity standpoint.

The vapor pressure data for a number of hydrated salts are given in Table II. In this table the vapor pressures have been expressed as the relative humidity at the given temperature. At atmospheric humidities lower than those given in the fourth column, the saturated solution will dry out leaving the crystalline hydrate; and this hydrate will decompose to the next lower hydrate, if one exists, at relative humidities lower than those given in the third column.

TABLE II

VAPOR PRESSURE OF SOME HYDRATED SALTS, AND OF THEIR SATURATED SOLUTIONS

Salt	Water in hydrate, %	Vapor pressure of hydrate expressed as relative humidity at given temperature (1), %	Vapor pressure of saturated solution expressed as relative humidity at given temperature (2), %	Remarks
NaClO ₃	0	—	75 (20° C.)	M.p. of hydrate, 29.9° C.
Ca(ClO ₃) ₂ · 2H ₂ O	14.8	—	—	
Ba(ClO ₃) ₂ · H ₂ O	5.6	—	—	
Na ₂ Cr ₂ O ₇ · 2H ₂ O	12.1	—	52 (20° C.)	
CaCl ₂ · 6H ₂ O	49.3	22 (25° C.)	31 (24.5° C.)	
Na ₂ CO ₃ · 10H ₂ O	62.9	76 (25° C.)	87 (24.5° C.)	M.p. of hydrate, 32-34° C.
Al ₂ (SO ₄) ₃ (NH ₄) ₂ SO ₄ · 24H ₂ O	47.6	23 (50° C.)	—	*Av. last two values given in tables.
Al ₂ (SO ₄) ₃ K ₂ SO ₄ · 24H ₂ O	45.5	26 (20° C.)	—	
Cr ₂ (SO ₄) ₃ K ₂ SO ₄ · 24H ₂ O	43.2	26 (30° C.)	—	
CuSO ₄ · 5H ₂ O	36.0	33 (20° C.)	94 (60° C.)	
MgCl ₂ · 6H ₂ O	53.1	4.6 (31.6° C.)	32 (25° C.)	
FeCl ₃ · 6H ₂ O	40.0	14 (15° C.)	—	
Na ₂ B ₄ O ₇ · 10H ₂ O	47.1	49 (20° C.)	—	
NaOH · H ₂ O	31.0	0.6 (25° C.)	—	
Na ₂ HPO ₄ · 12H ₂ O	60.3	78 (25° C.)*	—	
ZnCl ₂ (lowest hydrate)	—	3.6 (25° C.)	10 (20° C.)	
ZnSO ₄ · 7H ₂ O	43.8	57 (25° C.)	90 (20° C.)	

(1) Data obtained from Vol. VII, *Int. Crit. Tables*. (2) Data obtained from Vols. I and III, *Int. Crit. Tables*.

The available data on the properties of the oxidizing agents themselves are given first in Table II. Sodium chlorate does not form a hydrate and it forms a saturated solution only at relative humidities higher than 75%. The higher water content of calcium and barium chlorate is evident, but there are few data available concerning the vapor pressure of these hydrates. Sodium dichromate holds 12.1% of water, and will take up moisture to form a saturated solution at relative humidities higher than 52%. This, together with its higher equivalent weight, indicates that it should be less dangerous than sodium chlorate.

Calcium chloride and sodium carbonate are the two substances most commonly mixed with sodium chlorate in commercial herbicides, and the properties of these salts appear next in the table. Although the carbonate holds a higher percentage of water, it decomposes under ordinary atmospheric conditions, the saturated solution drying out at humidities below 87%, while the decahydrate itself decomposes at humidities below 76% of saturation. On the other hand, solutions of calcium chloride are stable at all relative humidities above 31%, and the hexahydrate exists at humidities as low as 22%.

The other salts listed in Table II represent some of the compounds tested as protecting agents in this investigation. The alums contain about 45% of water and decompose at relative humidities below about 26%. Magnesium chloride holds more water of crystallization than calcium chloride, and this hydrate is stable at lower humidities and higher temperatures than that of the calcium salt. These data indicate that magnesium chloride should be the best protecting agent of the chemicals listed in Table II. The remaining hydrated salts are less promising for they either hold a lower percentage of water or decompose at ordinary atmospheric humidities. Sulphuric acid would be one of the most effective reagents for retaining moisture under ordinary conditions, but unfortunately its admixture with sodium chlorate would result in the formation of chloric acid which decomposes readily, with a consequent reduction in the toxic properties of the herbicide. Many other reagents, commonly used for moisture absorption, are too expensive to be of practical value for this purpose.

The protecting action of these and other salts was determined in the manner previously described for testing the inflammability of sodium chlorate-organic matter mixtures. The salt to be tested was dissolved with the sodium chlorate, in various proportions, in the test solution. By using solutions of different concentrations it was also possible to vary the percentage of organic matter in the test material. Cloth (12-oz. duck) was the principal experimental substance. Initially dried leaves and filter paper were also included as test materials, but these were abandoned as it was found impossible to get quantitative results owing to their disintegration at various stages of the test. Both of these materials, however, appeared to be somewhat more combustible than cloth treated in a similar manner.

The first protecting salt tested was calcium chloride and the results obtained are presented in Table III. This table shows the proportion of anhydrous sodium chlorate used, and the concentration of the mixed salts in the test solution in percentage; *i.e.*, grams of salt mixture per 100 cc. of solution. Under the heading "Composition of test material" the amount of combustible material, salt, and water, have been expressed as a percentage of the whole. The percentage of salt includes both the sodium chlorate and calcium chloride but the percentage of sodium chlorate can be estimated, without serious error, from the proportion of this salt in the test solution.

Table III shows that all material tested at 11.5% relative humidity had a low moisture content and was hazardous. This is to be expected since

$\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ loses some of its water of crystallization at relative humidities below about 22%. At relative humidities higher than 31%, calcium chloride will exist in the form of a saturated solution, but there does not appear to be a break in the curve at this point, although the percentage of water in the test pieces increases steadily as the relative humidity is raised. Furthermore,

TABLE III
COMBUSTIBILITY OF NaClO_3 - CaCl_2 -CLOTH MIXTURES

Composition of salt mixture	Concentration of test solution, %	Relative humidity used for conditioning, %	Composition of test material			Result of combustion test
			Combustible material, %	Salt, %	Water, %	
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{CaCl}_2$	60*	11.5	48.2	46.3	5.5	Hazard
		29.0	42.6	40.8	16.6	No hazard
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{CaCl}_2$	40	11.5	62.0	32.6	5.4	Hazard
		29.0	58.8	29.3	11.9	No hazard
		43.0	55.0	28.0	17.0	No hazard
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{CaCl}_2$	20	29.0	73.6	19.1	7.3	Doubtful hazard
		43.0	70.8	18.6	10.6	Doubtful hazard
		53.0	67.6	18.2	14.2	No hazard
$\frac{2}{3} \text{NaClO}_3$ $\frac{1}{3} \text{CaCl}_2$	60	11.5	52.4	42.6	5.0	Hazard
		29.0	50.0	39.0	11.0	Doubtful hazard
		43.0	43.5	41.6	14.9	No hazard
$\frac{2}{3} \text{NaClO}_3$ $\frac{1}{3} \text{CaCl}_2$	45	11.5	60.4	35.7	3.9	Hazard
		29.0	56.9	34.4	8.7	Hazard
		43.0	52.7	33.1	14.2	Doubtful hazard
		53.0	52.0	30.5	17.5	No hazard
$\frac{2}{3} \text{NaClO}_3$ $\frac{1}{3} \text{CaCl}_2$	30	11.5	68.7	27.2	4.1	Hazard
		29.0	68.4	24.6	7.0	Hazard
		43.0	64.5	25.5	10.0	Doubtful hazard
		53.0	61.5	24.9	13.6	Doubtful hazard
$\frac{3}{4} \text{NaClO}_3$ $\frac{1}{4} \text{CaCl}_2$	40	29.0	63.1	30.2	6.7	Hazard
		43.0	60.3	30.1	9.6	Hazard
		53.0	58.3	29.1	12.6	Doubtful hazard
		61.0	56.0	28.4	15.6	No hazard
$\frac{3}{4} \text{NaClO}_3$ $\frac{1}{4} \text{CaCl}_2$	20	29.0	75.4	19.2	5.4	Hazard
		43.0	74.1	18.8	7.1	Hazard
		53.0	72.0	18.9	9.1	Doubtful hazard
		61.0	68.6	17.7	13.7	Ext. doubtful hazard
$\frac{4}{5} \text{NaClO}_3$ $\frac{1}{5} \text{CaCl}_2$	25	11.5	73.1	23.8	3.1	Hazard
		29.0	71.5	23.4	5.1	Hazard
		43.0	69.2	23.6	7.2	Hazard
		53.0	68.9	22.0	9.1	Hazard
		61.0	65.7	21.2	13.1	Doubtful hazard
$\frac{4}{5} \text{NaClO}_3$ $\frac{1}{5} \text{CaCl}_2$	12.5	43.0	81.2	12.4	6.4	Doubtful hazard
		53.0	79.8	12.0	8.2	Doubtful hazard
		61.0	77.6	11.6	10.8	No hazard

*Part of material undissolved.

test material classed as "hazard" and "doubtful hazard" at 29% relative humidity frequently shows no significant decrease in combustibility when the humidity is raised to 43%. Comparison of tests in the same group at the same relative humidity shows, as pointed out earlier, that with the exception of the lowest values the combustibility tends to increase as the percentage of the mixed salt, sodium chlorate and calcium chloride, in the test material decreases. The inflammability naturally increases as the proportion of calcium chloride in the sodium chlorate is reduced. The minimum amount of calcium chloride required for protection will depend on the atmospheric humidity to which the herbicide is exposed. Thus equal parts of sodium chlorate and anhydrous calcium chloride can be considered safe at all relative humidities above 43%; two parts of sodium chlorate and one part of anhydrous calcium chloride will be comparatively safe at all humidities above about 53%, while four parts of sodium chlorate to one of calcium chloride will be safe only when the relative humidity is above about 60%. The effect of these large proportions of calcium chloride on the toxic properties of the herbicide will be discussed later.

TABLE IV
COMBUSTIBILITY OF NaClO_3 - MgCl_2 -CLOTH MIXTURES

Com- position of salt mixture	Con- centration of test solution, %	Relative humidity used for con- ditioning, %	Composition of test material			Result of combustion test
			Com- bustible material, %	Salt, %	Water, %	
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{MgCl}_2$	60	11.5	41.2	49.0	9.8	No hazard
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{MgCl}_2$	40	11.5	55.0	37.0	8.0	No hazard
		29.0	51.2	34.0	14.8	No hazard
		43.0	49.2	32.0	18.8	No hazard
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{MgCl}_2$	20	11.5	72.7	22.0	5.3	Doubtful hazard
		29.0	72.2	19.4	8.4	No hazard
$\frac{2}{3} \text{NaClO}_3$ $\frac{1}{3} \text{MgCl}_2$	45	11.5	55.8	37.5	6.7	Doubtful hazard
		29.0	53.8	35.0	11.2	No hazard
$\frac{2}{3} \text{NaClO}_3$ $\frac{1}{3} \text{MgCl}_2$	30	11.5	67.6	27.6	4.8	Doubtful hazard
		29.0	63.0	28.4	8.6	Doubtful hazard
		43.0	61.7	26.3	12.0	No hazard
$\frac{3}{4} \text{NaClO}_3$ $\frac{1}{4} \text{MgCl}_2$	40	29.0	61.5	31.3	7.2	Doubtful hazard
		43.0	60.6	29.6	9.8	Doubtful hazard
		53.0	58.1	28.7	13.2	Doubtful hazard
$\frac{3}{4} \text{NaClO}_3$ $\frac{1}{4} \text{MgCl}_2$	20	29.0	71.8	22.0	6.2	Hazard
		43.0	69.9	22.4	7.7	Doubtful hazard
$\frac{4}{5} \text{NaClO}_3$ $\frac{1}{5} \text{MgCl}_2$	25	29.0	73.0	22.0	5.0	Hazard
		43.0	71.6	21.3	7.1	Doubtful hazard
		53.0	66.0	24.5	9.5	Doubtful hazard
$\frac{4}{5} \text{NaClO}_3$ $\frac{1}{5} \text{MgCl}_2$	12.5	29.0	81.0	13.8	5.2	Doubtful hazard
		43.0	80.3	13.0	6.7	Doubtful hazard
		53.0	78.9	12.6	8.5	No hazard

Magnesium chloride was the next salt considered since the data presented in Table II indicated that it should be the best protecting agent of the hydrated salts listed in that table. The data are presented in Table IV in the same form as in Table III. It is again evident that, with the exception of the mixtures containing the smallest amount of the mixed salt, the fire hazard decreases as the proportion of mixed herbicide increases. These experiments bear out the earlier indications that magnesium chloride is a better protecting agent than calcium chloride. Mixtures of equal parts of sodium chlorate and anhydrous magnesium chloride are safe at 29% relative humidity, and even mixtures of two parts of sodium chlorate to one of magnesium chloride are relatively safe at this humidity, and entirely safe above 43%. Mixtures containing lower proportions of magnesium chloride are a doubtful hazard at 53% humidity, the highest value used in this experiment.

Mixtures of sodium chlorate and sodium carbonate are used as commercial herbicides, and this latter salt was the next to receive consideration. The results obtained are presented in Table V. They show that sodium carbonate

TABLE V
COMBUSTIBILITY OF NaClO_3 - Na_2CO_3 -CLOTH, AND NaClO_3 - NaOH -CLOTH MIXTURES

Composition of salt mixture	Concentration of test solution, %	Relative humidity used for conditioning, %	Composition of test material			Result of combustion test	Remarks
			Combustible material, %	Salt, %	Water, %		
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{Na}_2\text{CO}_3$	40	11.5	73.6	24.4	2.0	Hazard	Spontaneous spread without flame but with numerous sparks Some ppt. in soln.
		29.0	71.3	26.6	2.1	Hazard	
		43.0	69.4	24.7	5.9	Hazard	
		53.0	68.4	24.0	7.6	Doubtful hazard	
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{Na}_2\text{CO}_3$	60	11.5	58.2	40.4	1.4	Hazard	Quite heavy ppt. No flame (sparks)
		29.0	56.8	40.4	2.8	Hazard	
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{Na}_2\text{CO}_3$	30	11.5	67.9	30.3	1.8	Hazard	Slight ppt. No flame but sparks
		43.0	66.7	27.6	5.7	Hazard	
		53.0	65.7	27.3	7.0	Hazard	
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{NaOH}$	40	11.5	—	—	(2.7)	Hazard	Test pieces swelled in this soln., and disintegrated, making dry weight unreliable
		29.0	—	—	(11.1)	No hazard	
		43.0	—	—	(14.1)	No hazard	
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{NaOH}$	20	11.5	(75.7)	(21.7)	(2.6)	Hazard	Weights given but are not considered reliable due to swelling
		29.0	(69.6)	(21.8)	(8.6)	Doubtful hazard	
		43.0	(68.6)	(21.6)	(9.8)	No hazard	
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{NaOH}$	30	11.5	(68.4)	(30.0)	(1.6)	Hazard	Weights given but are not considered reliable due to swelling
		29.0	(66.3)	(26.9)	(6.8)	Hazard	
		43.0	(64.5)	(26.2)	(9.3)	Hazard	
		53.0	(62.8)	(26.4)	(10.8)	Hazard	

retains very little moisture and is of little value as a protecting agent. These experiments covered mixtures composed of one-half and one-third anhydrous sodium carbonate only, and as these were a distinct hazard, lower proportions of carbonate were not tested.

Although sodium carbonate was of little value as a protecting agent, judging from the rapid spread of the burnt area, it did have an effect on the inflammability, as none of the pieces burned with a free flame. However, in view of the large number of sparks developed, and the rapid spread of the burnt area, the lack of flame cannot be considered a practical advantage. In any event, sodium carbonate appears to be inferior to calcium or magnesium chloride for admixture with sodium chlorate as a safeguard against fire.

The data given in Table II show that sodium hydroxide retains about 31% of water at very low relative humidities. Under atmospheric conditions, however, most of this substance would be converted to sodium carbonate. Its value as a protecting agent was nevertheless determined using the method employed in testing the other salts. The data obtained are presented in Table V. The test material swelled considerably while being treated in the caustic-chlorate solutions, and a considerable amount of carbonate was formed on the material during subsequent conditioning. The quantitative data, concerning the composition of the material, are consequently only approximate. The combustion test shows that mixtures of one-half sodium chlorate and one-half sodium hydroxide are comparatively safe at relative humidities above 29%, and completely safe at humidities above 43%. Mixtures containing only one-third sodium hydroxide, however, are hazardous at all humidities up to 53%, the highest value tested. Comparison of the protecting action of sodium carbonate, formed on the material by treatment in alkali and subsequent exposure to the air, with that of sodium carbonate applied in solution, suggests that the former is more effective. It must be remembered, however, that the proportion of carbonate is somewhat higher in the material treated with sodium hydroxide than in the material treated with sodium carbonate directly.

Table VI shows the results obtained with three alums and with chromium nitrate. It was found impossible to make 40% solutions of salt mixtures containing one-half aluminium potassium or aluminium ammonium alum, as some of the material would not dissolve. The solution resulting from an attempt to prepare a 40% concentration was tested, however, and it is evident that the amount of alum which did dissolve had little protective action. Mixtures composed of two-thirds sodium chlorate and one-third alum were also hazardous although they dissolved to form a 30% solution. As these salts showed no promise as protecting agents no further tests were made.

The results obtained with chrome alum show that none of the material was dangerous even at relative humidities of 11.5%. It was observed, however, that the test material gained but little weight as the humidity was increased, in fact in some cases weight was lost. Furthermore, the tensile strength of

the material was reduced and tests for the chlorate ion showed that most of it had disappeared. These observations suggested that the chromium ion tended to catalyze the oxidation of the organic material by the sodium chlorate.

TABLE VI

COMBUSTIBILITY OF $\text{NaClO}_3\text{-Al}_2(\text{SO}_4)_3\text{K}_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$ -CLOTH, $\text{NaClO}_3\text{-Al}_2(\text{SO}_4)_3(\text{NH}_4)_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$ -CLOTH, $\text{NaClO}_3\text{-Cr}_2(\text{SO}_4)_3(\text{NH}_4)_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$ -CLOTH, AND $\text{NaClO}_3\text{-Cr}(\text{NO}_3)_3\text{-CLOTH MIXTURES}$

Composition of salt mixture	Concentration of test solution, %	Relative humidity used for conditioning, %	Composition of test material			Result of combustion test	Remarks
			Combustible material, %	Salt, %	Water, %		
$\left. \begin{array}{l} \frac{1}{3} \text{NaClO}_3 \\ \frac{1}{3} \text{Al}_2(\text{SO}_4)_3\text{K}_2\text{SO}_4 \cdot 24\text{H}_2\text{O} \end{array} \right\}$	40	11.5	71.8	26.2	2.0	Hazard	Slight ppt.
		29.0	70.6	25.4	4.0	Hazard	
		43.0	68.8	25.7	5.5	Hazard	
$\left. \begin{array}{l} \frac{1}{3} \text{NaClO}_3 \\ \frac{1}{3} \text{Al}_2(\text{SO}_4)_3\text{K}_2\text{SO}_4 \cdot 24\text{H}_2\text{O} \end{array} \right\}$	30	11.5	74.4	24.9	0.7	Hazard	
		29.0	73.2	22.6	4.2	Hazard	
		43.0	71.7	22.6	5.7	Hazard	
$\left. \begin{array}{l} \frac{1}{3} \text{NaClO}_3 \\ \frac{1}{3} \text{Al}_2(\text{SO}_4)_3(\text{NH}_4)_2\text{SO}_4 \cdot 24\text{H}_2\text{O} \end{array} \right\}$	40	11.5	73.6	24.4	2.0	Hazard	Heavy precipitate
		29.0	72.0	22.5	5.5	Hazard	
		43.0	69.5	(24.9)	(5.6)	Doubtful hazard	
$\left. \begin{array}{l} \frac{1}{3} \text{NaClO}_3 \\ \frac{1}{3} \text{Al}_2(\text{SO}_4)_3(\text{NH}_4)_2\text{SO}_4 \cdot 24\text{H}_2\text{O} \end{array} \right\}$	30	11.5	76.8	21.0	2.2	Hazard	
		29.0	74.6	21.4	4.0	Hazard	
		43.0	72.4	22.0	5.6	Doubtful hazard	
$\left. \begin{array}{l} \frac{1}{3} \text{NaClO}_3 \\ \frac{1}{3} \text{Cr}_2(\text{SO}_4)_3(\text{NH}_4)_2\text{SO}_4 \cdot 24\text{H}_2\text{O} \end{array} \right\}$	40	11.5	(71.3)	(26.8)	(1.9)	No hazard	Pieces lost weight, and had much lower tensile strength after washing and standing
		29.0	(71.5)	(25.0)	(3.5)	No hazard	
		43.0	(71.0)	(24.8)	(4.2)	No hazard	
$\left. \begin{array}{l} \frac{1}{3} \text{NaClO}_3 \\ \frac{1}{3} \text{Cr}_2(\text{SO}_4)_3(\text{NH}_4)_2\text{SO}_4 \cdot 24\text{H}_2\text{O} \end{array} \right\}$	30	11.5	(76.6)	(21.4)	(2.0)	Doubtful hazard	Pieces lost weight, and had much lower tensile strength after washing and standing
		29.0	(74.8)	(21.8)	(3.4)	Doubtful hazard	
		43.0	(73.2)	(22.5)	(4.3)	No hazard	
$\left. \begin{array}{l} \frac{1}{3} \text{NaClO}_3 \\ \frac{1}{3} \text{Cr}(\text{NO}_3)_3 \end{array} \right\}$	40	11.5	(67.3)	(29.6)	(3.1)	Doubtful hazard	Pieces rotten
		29.0	(67.4)	(27.5)	(5.1)	No hazard	
		43.0	(66.5)	(27.4)	(6.1)	No hazard	
$\left. \begin{array}{l} \frac{1}{3} \text{NaClO}_3 \\ \frac{1}{3} \text{Cr}(\text{NO}_3)_3 \end{array} \right\}$	30	11.5	(72.9)	(24.6)	(2.5)	Doubtful hazard	Pieces rotten
		29.0	(71.6)	(25.1)	(3.3)	Doubtful hazard	
		43.0	(70.6)	(24.0)	(5.4)	Doubtful hazard	
$\left. \begin{array}{l} \frac{1}{3} \text{NaClO}_3 \\ \frac{1}{3} \text{Cr}(\text{NO}_3)_3 \end{array} \right\}$	25	11.5	(74.8)	(23.0)	(2.2)	Hazard	
		29.0	(74.6)	(21.5)	(3.9)	Doubtful hazard	
		43.0	(74.8)	(21.0)	(4.2)	Doubtful hazard	

Tests were made with chromium nitrate to check this point, with similar results. Determination of the hydrogen ion concentration in these solutions showed that they were of pH approximately 2.0 and as this degree of acidity would cause the formation of chloric acid, which decomposes in the presence of organic matter, the disappearance may be explained by the acidity of the solution rather than by the presence of the chromium. In any event, such salts could not be used as protecting agents in chlorate herbicides, since they destroy the toxic compound.

The results obtained with copper sulphate, ferric chloride, and aluminium chloride, are presented in Table VII. It seemed from the point of view of

TABLE VII
COMBUSTIBILITY OF $\text{NaClO}_3\text{-CuSO}_4\text{-CLOTH}$, $\text{NaClO}_3\text{-FeCl}_3\text{-CLOTH}$, AND
 $\text{NaClO}_3\text{-AlCl}_3\text{-CLOTH}$ MIXTURES

Composition of salt mixture	Concentration of test solution, %	Relative humidity used for conditioning, %	Composition of test material			Result of combustion test	Remarks
			Combustible material, %	Salt, %	Water, %		
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{CuSO}_4$	40	11.5	60.0	34.0	6.0	Hazard	
		29.0	62.7	32.4	4.9	Hazard	
		43.0	60.3	33.1	6.6	Hazard	
		53.0	64.8	26.5	8.7	Hazard	
		61.0	64.2	27.5	8.3	Hazard	
		71.0	Not weighed			No hazard	
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{CuSO}_4$	30	11.5	70.5	27.0	2.5	Hazard	
		29.0	69.3	26.0	4.7	Hazard	
		43.0	67.4	26.0	6.6	Hazard	
		61.0	65.6	26.0	8.4	Hazard	
		71.0	Not weighed			Doubtful hazard	
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{FeCl}_3$	40	11.5	(64.3)	(32.1)	(3.6)	Doubtful hazard	Soln. decomposed having strong odor of chlorine
		29.0	(62.5)	(26.6)	(10.9)	Doubtful hazard	
		43.0	(60.8)	(30.2)	(9.0)	No hazard	
	30	11.5	(71.6)	(25.5)	(2.9)	Doubtful hazard	Soln. decomposed having strong odor of chlorine
		29.0	(69.3)	(25.8)	(4.9)	Doubtful hazard	
		43.0	(70.8)	(23.2)	(6.0)	Doubtful hazard	
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{AlCl}_3$	40	11.5	(58.4)	(38.8)	(2.8)	No hazard	Soln. decomposed giving off Cl_2 gas and leaving a white ppt.
		29.0	(57.8)	(37.2)	(5.0)	No hazard	
	30	11.5	(70.0)	(26.4)	(3.6)	Doubtful hazard	Soln. decomposed giving off Cl_2 gas but solution remained clear
		29.0	(68.4)	(25.6)	(6.0)	Doubtful hazard	
		43.0	(66.7)	(25.1)	(8.2)	No hazard	

herbicidal value that salts such as copper sulphate, which are quite toxic to plants, might be used in a mixed herbicide more profitably than salts such as calcium chloride which are less toxic. A herbicide composed of equal parts of sodium chlorate and copper sulphate, however, was found in these tests to be hazardous at relative humidities as high as 61%. These results show that copper sulphate is of little value in reducing the inflammability of sodium chlorate-organic matter mixtures even when it is present in large proportions.

Ferric chloride and aluminium chloride are both hydrated salts and were therefore tested for their protective action. When these substances were dissolved in the solution containing the sodium chlorate, decomposition set in immediately with the evolution of chlorine. Both salts made the solution quite acidic. Further decomposition of the chlorate took place after the test pieces had been treated, and the reduced combustibility of material treated in solutions containing the above salts can be explained by the disappearance of the chlorate ion. In consequence neither ferric nor aluminium chloride can be used as protecting agents in chlorate herbicides.

The next series of substances tested consists of salts, which in addition to their capacity for retaining moisture are known to exert a definite fire-preventing action. These include sodium borate, sodium stannate, sodium and ammonium acid phosphate, zinc chloride and zinc sulphate. Ammonium phosphate and zinc chloride, applied under pressure, have been used quite extensively for fireproofing wood. Sodium stannate has been used for fireproofing cloth, the material being treated with a sodium stannate solution first, and the metallic ion subsequently precipitated on the material by treatment with another solution. Obviously such treatments cannot be employed to lower the combustibility of organic material contaminated with sodium chlorate. The only test of these materials applicable to field conditions is to mix the reagent with the sodium chlorate and test its effect on the combustibility when applied in this manner.

The results obtained with the above salts are presented in Table VIII. Sodium borate and the two phosphate salts were too insoluble at ordinary temperatures in the chlorate solutions to permit high proportions of these salts to be used. Even in the proportions used considerable amounts of undissolved material remained and it is evident that these substances exerted little protective action. Sodium stannate like sodium carbonate seemed to arrest the formation and spread of flame, but the burnt area spread rapidly without flame and the material was considered quite hazardous at low humidities. The zinc salts in addition to their water-retaining and fire-preventing properties, are quite good herbicides. In these tests, however, they seemed not to affect the combustibility to any extent, and are consequently of little value for admixture with sodium chlorate with the object of reducing the fire risk.

Soluble arsenicals and ammonium thiocyanate are known to be effective herbicides, and although none of these salts would be expected to reduce the combustibility of organic material significantly, they could form a much larger

TABLE VIII

COMBUSTIBILITY OF $\text{NaClO}_3\text{-Na}_2\text{B}_4\text{O}_7\text{-CLOTH}$, $\text{NaClO}_3\text{-Na}_2\text{SnO}_3\text{-CLOTH}$, $\text{NaClO}_3\text{-Na}_2\text{HPO}_4\text{-CLOTH}$, $\text{NaClO}_3\text{-ZnCl}_2\text{-CLOTH}$ AND $\text{NaClO}_3\text{-ZnSO}_4\text{-CLOTH}$ MIXTURES

Composition of salt mixture	Concentration of test solution, %	Relative humidity used for conditioning, %	Composition of test material			Results of combustion test	Remarks
			Combustible material, %	Salt, %	Water, %		
{ NaClO_3 $\text{Na}_2\text{B}_4\text{O}_7$ }	30	11.5	74.8	23.5	1.7	Hazard	Heavy pp't. from this test sol'n.
		29.0	74.1	22.2	3.7	Hazard	
		43.0	73.5	21.0	5.5	Hazard	
{ NaClO_3 Na_2SnO_3 }	40	11.5	61.7	35.8	2.5	Hazard	Slight precipitate
		29.0	60.0	35.2	4.8	Doubtful hazard	
		43.0	59.6	33.5	6.9	Doubtful hazard	
{ NaClO_3 Na_2SnO_3 }	30	11.5	69.7	27.6	2.7	Doubtful hazard	Slight precipitate
		29.0	68.6	25.6	5.8	Doubtful hazard	
		43.0	67.8	25.2	7.0	Doubtful hazard	
{ NaClO_3 Na_2HPO_4 }	30	11.5	72.8	25.4	1.8	Hazard	Heavy precipitate
		29.0	70.5	25.0	4.5	Hazard	
		43.0	69.6	25.4	5.0	Hazard	
{ NaClO_3 $(\text{NH}_4)_2\text{HPO}_4$ }	30	11.5	73.7	25.0	1.3	Hazard	Heavy precipitate from this test solution
		29.0	72.3	24.6	3.1	Hazard	
		43.0	72.3	22.8	4.9	Hazard	
{ NaClO_3 ZnCl_2 }	40	11.5	64.4	32.6	3.0	Hazard	
		29.0	63.3	31.0	5.7	Hazard	
{ NaClO_3 ZnCl_2 }	60	11.5	57.2	40.4	2.4	Hazard	
		29.0	57.7	37.4	4.9	Hazard	
{ NaClO_3 ZnCl_2 }	30	11.5	72.1	25.6	2.3	Hazard	
		29.0	70.9	24.5	4.6	Hazard	
{ NaClO_3 ZnSO_4 }	40	11.5	64.5	33.8	1.7	Hazard	ZnSO ₄ seemed to cut down intensity of flame but did not remove the fire risk
		29.0	63.1	32.8	4.1	Hazard	
		43.0	63.2	32.8	4.0	Doubtful hazard	
{ NaClO_3 ZnSO_4 }	30	11.5	70.0	28.8	1.2	Hazard	
		29.0	68.7	27.4	3.9	Hazard	
		43.0	67.3	27.8	4.9	Hazard	

proportion of the herbicide without significantly reducing the toxicity. The protective action of arsenic pentoxide, sodium arsenite, sodium arsenate and ammonium thiocyanate, was therefore tested and the results obtained are presented in Table IX. Arsenic pentoxide and ammonium thiocyanate made the sodium chlorate solution quite acidic and partial decomposition of the chlorate resulted. This effect eliminates the possibility of using them as

protective agents. Mixtures composed of one-half sodium arsenite or sodium arsenate with sodium chlorate, when applied to organic material, remained a doubtful fire risk at relative humidities of 43%. The practical value of such mixtures is not likely to be great, in view of the fact that, although the combustibility is reduced as compared with pure sodium chlorate, a fire risk still exists, and in addition the herbicide has been made highly poisonous to live-stock by the addition of the arsenical.

TABLE IX

COMBUSTIBILITY OF $\text{NaClO}_3\text{-As}_2\text{O}_3\text{-CLOTH}$, $\text{NaClO}_3\text{-Na}_2\text{HAsO}_4\text{-CLOTH}$, $\text{NaClO}_3\text{-Na}_2\text{HAsO}_4\text{-CLOTH}$, AND $\text{NaClO}_3\text{-NH}_4\text{CNS-CLOTH MIXTURES}$

Composition of salt mixture	Concentration of test solution, %	Relative humidity used for conditioning, %	Composition of test material			Results of combustion test	Remarks
			Combustible material, %	Salt, %	Water, %		
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{As}_2\text{O}_3$	40	11.5	(67.1)	(30.8)	(2.1)	Hazard	All of these solutions decomposed as shown by the extreme bleaching and reduction in strength of the test pieces
		29.0	(65.6)	(30.3)	(4.1)	Hazard	
		43.0	(65.5)	(28.8)	(5.7)	Doubtful hazard	
		53.0	(64.5)	(28.2)	(7.3)	Doubtful hazard	
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{As}_2\text{O}_3$	20	11.5	(80.8)	(17.2)	(2.0)	No hazard	(No precipitate in these mixtures)
		29.0	(78.6)	(17.2)	(4.2)	No hazard	
		43.0	(76.9)	(17.3)	(5.8)	No hazard	
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{As}_2\text{O}_3$	30	11.5	(71.8)	(26.4)	(1.8)	Hazard	
		29.0	(70.8)	(25.4)	(3.8)	Hazard	
		43.0	(70.5)	(24.6)	(4.9)	Hazard	
		53.0	(70.6)	(23.2)	(6.2)	Hazard	
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{Na}_2\text{HAsO}_4$	20	11.5	82.2	15.4	2.4	Hazard	
		29.0	79.8	14.9	5.3	Doubtful hazard	
		43.0	78.6	14.3	7.1	Doubtful hazard	
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{Na}_2\text{HAsO}_4$	30	11.5	75.4	22.4	2.2	Hazard	
		29.0	73.1	22.0	4.9	Hazard	
		43.0	71.5	22.2	6.3	Hazard	
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{Na}_2\text{HAsO}_4$	40	29.0	65.7	30.2	4.1	Hazard	
		43.0	62.6	27.3	10.1	Doubtful hazard	
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{Na}_2\text{HAsO}_4$	30	11.5	72.3	25.8	1.9	Hazard	
		29.0	70.6	24.5	4.9	Hazard	
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{NH}_4\text{CNS}$	30	11.5	71.0	27.5	1.5	Hazard	Solution clear at first but a heavy precipitate came out on standing. Slight odor of Cl_2
		29.0	69.3	27.2	3.5	Hazard	
		43.0	69.7	25.4	4.9	Hazard	

Experiments with Hydrated Chlorates

The experiments of the previous section show that any of the salts tested would have to be added to sodium chlorate in large proportions in order to reduce significantly the fire hazard accompanying the use of this substance. Furthermore, the salts most effective in reducing the combustibility are relatively non-toxic to plants and their addition to sodium chlorate will reduce the herbicidal power to a degree roughly proportional to the reduction in chlorate content. This consideration led to studies on another possible method of reducing the fire risk, namely, the application of the toxic chlorate ion in the form of one of its hydrated salts, such as barium or calcium chlorate.

Calcium chlorate carries two molecules of water of hydration, equivalent to 14.8% of the weight of the hydrated salt, and contains 68.8% chlorate ion calculated on the same basis. In comparison sodium chlorate carries no water of hydration, and is 78.4% chlorate. The herbicidal power of calcium chlorate, based on percentage chlorate, should therefore be somewhat lower than that of sodium chlorate.

Combustibility tests were made on cloth treated in 10, 20 and 30% solutions of calcium chlorate. The data obtained are presented in Table X. They show that material treated with 10% calcium chlorate becomes a doubtful hazard at a relative humidity of 43%, and is not completely safe until the humidity is raised to 61%. Material treated with 20% and 30% solutions of calcium chlorate become a doubtful hazard at 53% relative humidity, and the combustibility is not significantly changed by raising the humidity to

TABLE X
COMBUSTIBILITY OF $\text{Ca}(\text{ClO}_3)_2$ -CLOTH MIXTURES

Composition of salt mixture	Concentration of test solution, %	Relative humidity used for conditioning, %	Composition of test material			Results of combustion test
			Combustible material, %	Salt, %	Water, %	
$\text{Ca}(\text{ClO}_3)_2$	30	11.5	70.4	25.2	4.4	Hazard
		29.0	64.5	25.0	10.5	Hazard
		43.0	62.8	24.0	13.2	Hazard
		53.0	59.7	21.8	18.5	Doubtful hazard
		61.0	58.2	21.4	20.4	Doubtful hazard
$\text{Ca}(\text{ClO}_3)_2$	20	11.5	75.5	20.4	4.1	Hazard
		29.0	73.9	18.1	8.0	Hazard
		43.0	72.7	17.3	10.0	Hazard
		53.0	70.6	16.7	12.7	Doubtful hazard
		61.0	68.5	16.2	15.3	Doubtful hazard
$\text{Ca}(\text{ClO}_3)_2$	10	11.5	84.4	12.2	3.4	Hazard
		29.0	82.2	11.7	6.1	Hazard
		43.0	80.7	11.5	7.8	Doubtful hazard
		53.0	78.9	11.4	9.7	Doubtful hazard
		61.0	77.5	11.0	11.5	No hazard

61%. As material treated with sodium chlorate solutions is a definite fire hazard at all relative humidities below 75%, it is evident that calcium chlorate is the safer chemical for general use. The observed difference in inflammability between these two salts must be attributed to the water content of the calcium salt rather than to the lower percentage of chlorate, since

TABLE XI
COMBUSTIBILITY OF $\text{Ba}(\text{ClO}_3)_2$ -CLOTH, $\text{Ba}(\text{ClO}_3)_2$ - CaCl_2 -CLOTH, AND
 $\text{Ba}(\text{ClO}_3)_2$ - MgCl_2 -CLOTH MIXTURES

Composition of salt mixture	Concentration of test solution, %	Relative humidity used for conditioning, %	Composition of test material			Result of combustion test
			Combustible material, %	Salt, %	Water, %	
$\text{Ba}(\text{ClO}_3)_2$	30	11.5	71.3	26.8	1.9	Hazard
		29.0	70.0	26.2	3.8	Hazard
		43.0	69.6	26.0	4.4	Hazard
		53.0	68.5	25.4	6.1	Hazard
$\text{Ba}(\text{ClO}_3)_2$	20	11.5	79.0	18.9	2.1	Hazard
		29.0	77.2	18.8	4.0	Hazard
		43.0	76.2	18.9	4.9	Doubtful hazard
		53.0	75.8	18.0	6.2	Doubtful hazard
		61.0	75.1	17.8	7.1	Doubtful hazard
$\text{Ba}(\text{ClO}_3)_2$	10	11.5	85.9	12.2	1.9	Hazard
		29.0	84.4	11.6	4.0	Doubtful hazard
		43.0	83.5	11.7	4.8	Doubtful hazard
		53.0	82.4	11.5	6.1	No hazard
$\frac{2}{3} \text{Ba}(\text{ClO}_3)_2$ $\frac{1}{3} \text{CaCl}_2$	40*	11.5	63.3	31.8	4.9	No hazard
		29.0	58.3	28.5	13.2	No hazard
		43.0	55.8	28.1	16.1	No hazard
$\frac{1}{2} \text{Ba}(\text{ClO}_3)_2$ $\frac{1}{2} \text{CaCl}_2$	20	11.5	75.5	20.2	4.3	Doubtful hazard
		29.0	71.8	18.9	9.3	No hazard
		43.0	69.0	19.2	11.8	No hazard
$\frac{2}{3} \text{Ba}(\text{ClO}_3)_2$ $\frac{1}{3} \text{CaCl}_2$	30	11.5	68.6	27.5	3.9	Doubtful hazard
		29.0	65.0	25.2	9.8	No hazard
		43.0	63.4	26.1	10.5	No hazard
$\frac{3}{4} \text{Ba}(\text{ClO}_3)_2$ $\frac{1}{4} \text{CaCl}_2$	15	11.5	78.4	15.4	6.2	Doubtful hazard
		29.0	77.5	16.3	6.2	Doubtful hazard
		43.0	76.0	15.1	8.9	Doubtful hazard
$\frac{4}{5} \text{Ba}(\text{ClO}_3)_2$ $\frac{1}{5} \text{CaCl}_2$	25	11.5	72.2	24.6	3.2	Doubtful hazard
		29.0	69.3	24.9	5.8	Doubtful hazard
		43.0	69.0	23.0	8.0	Doubtful hazard
$\frac{3}{4} \text{Ba}(\text{ClO}_3)_2$ $\frac{1}{4} \text{MgCl}_2$	15	11.5	80.6	15.8	3.6	Doubtful hazard
		29.0	79.1	15.2	5.7	Doubtful hazard
		43.0	76.8	15.4	7.8	No hazard
$\frac{4}{5} \text{Ba}(\text{ClO}_3)_2$ $\frac{1}{5} \text{MgCl}_2$	25	11.5	72.6	24.2	3.2	Doubtful hazard
		29.0	70.5	23.8	5.7	Doubtful hazard
		43.0	69.7	22.7	7.6	Doubtful hazard

*Slight precipitation.

material treated in 20% and 30% solutions of calcium chlorate are less hazardous than material treated in 10% and 20% solutions of sodium chlorate respectively. The contribution of the water carried by the hydrated salt to the total water content of the test pieces is indicated in the sixth column of Table X by the different percentages of water corresponding to the different concentrations of test solution. The fact remains, however, that calcium chlorate is quite dangerous at all ordinary atmospheric humidities. As calcium chlorate is difficult to prepare, and would probably prove too expensive for use as a herbicide, no further studies were made on this salt.

Barium chlorate contains only one molecule of water of hydration, equal to 5.6% of the weight of the hydrate. The percentage of chlorate ion in this compound is 51.8. Combustibility tests were conducted on cloth treated with 10, 20 and 30% solutions of this salt, and the results obtained are presented in Table XI. The data show that material treated with barium chlorate is less combustible than material treated with sodium chlorate, but in spite of its lower chlorate content, barium chlorate renders organic material more combustible than calcium chlorate. The lower hydration of this salt compared with calcium chlorate is also evident from a comparison of column 6, Tables X and XI. Barium chlorate would therefore be rather dangerous to use alone as a herbicide.

The feasibility of adding other salts to barium chlorate in order to reduce the fire risk was next investigated. Calcium and magnesium chlorides were used as these were found, in the previous experiments, to be most effective in reducing the fire risk involved in the use of sodium chlorate as a herbicide. Various proportions of these salts were tested and the results obtained are given in Table XI. The reduced combustibility and increased moisture content of material treated with these mixed herbicides in comparison with those treated with pure barium chlorate solutions is evident from the data. Herbicide mixtures composed of two-thirds barium chlorate and one-third of either anhydrous calcium or magnesium chloride will be entirely safe at ordinary atmospheric humidities.

Experiments with Sodium Dichromate

Sodium dichromate forms a major part of at least one commercial herbicide and preliminary tests (2) of its toxicity to plants suggest that it may have valuable herbicidal properties. As it is also a strong oxidizing agent, some tests were conducted to determine to what extent its use was accompanied by fire risk. Sodium dichromate-organic matter mixtures should be less hazardous than chlorate-organic matter mixtures since this dichromate salt carries two molecules of water of crystallization and has a greater equivalent weight.

The technique employed in these tests was the same as that previously described. The cloth test pieces were treated in sodium dichromate solutions of 10, 20, 30, 40 and 50% concentrations, and filter paper was treated in

solutions of 10, 30 and 50% concentrations. The amount of sodium dichromate taken up from these solutions by the two test materials is shown in Fig. 4 A. This shows again a linear relation similar to that obtained with sodium chlorate solutions (Fig. 3 A).

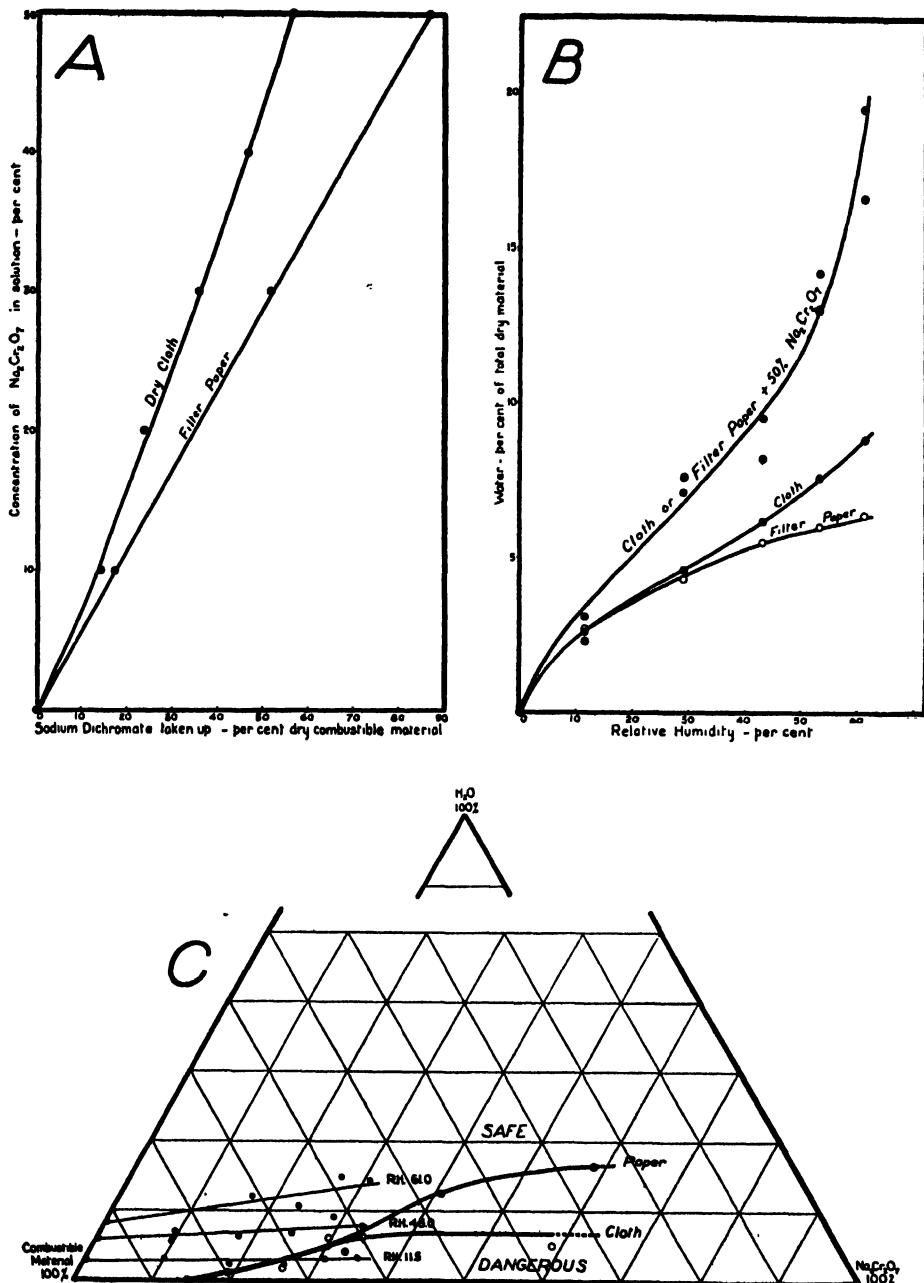


FIG. 4. Properties of test materials treated with sodium dichromate solutions.

The amount of moisture taken up by untreated cloth and filter paper, and by the same material treated with a 50% solution of sodium dichromate is shown in Fig. 4 B. As the dichromate salt takes up two molecules of water of crystallization, the treated materials took up more moisture than the untreated. This is the opposite behavior from that of test pieces treated with sodium chlorate which, at vapor pressures less than that of a saturated solution, took up less moisture than untreated material. (Fig. 3 B).

A saturated solution of sodium dichromate was found, in these experiments, to have a vapor pressure equivalent to a relative humidity of 53% at 25° C. Organic material containing sodium dichromate will therefore take up sufficient moisture to dissolve the salt when the humidity is above 53%. In these experiments the test pieces were conditioned, in succession, at relative humidities of 0, 11.5, 29, 43, 53 and 61%. The composition of the test pieces conditioned at 11.5, 43, and 61% relative humidity is shown in Fig. 4 C, the construction of which is similar to that of Fig. 3 C. The values at 61% relative humidity are not considered very reliable as some of the saturated solution may have drained off the material before weighing.

On determining the combustibility of the test materials treated with sodium dichromate, it was found that pieces soaked in 10 and 20% solutions were not hazardous even at relative humidities as low as 11.5%. None of the test materials, including filter paper soaked in a 50% solution, could be considered hazardous at a relative humidity of 43%. The complete results are shown in the triangular diagram, Fig. 4 C. The data shown on this chart were obtained in the same manner as those described under the tests on sodium chlorate, a few test pieces being soaked in saturated sodium dichromate in order to extend the range of the curves.

Comparison of Fig. 4 C with Fig. 3 C shows that sodium dichromate-organic matter mixtures are not nearly as combustible as similar mixtures

TABLE XII

COMBUSTIBILITY OF $\text{Na}_2\text{Cr}_2\text{O}_7\text{-CaCl}_2\text{-CLOTH}$, AND $\text{NaClO}_3\text{-Na}_2\text{Cr}_2\text{O}_7\text{-CLOTH}$ MIXTURES

Composition of salt mixture	Concentration of test solution, %	Relative humidity used for conditioning, %	Composition of test material			Results of combustion test
			Combustible material, %	Salt, %	Water, %	
$\frac{1}{2} \text{Na}_2\text{Cr}_2\text{O}_7$ $\frac{1}{2} \text{CaCl}_2$	60	11.5 29.0	56.9 55.1	40.9 38.7	2.2 6.2	Doubtful hazard No hazard
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{Na}_2\text{Cr}_2\text{O}_7$	40	11.5 29.0 43.0	64.8 61.2 61.5	32.8 33.1 32.6	2.4 5.7 5.9	Hazard Hazard Hazard
$\frac{1}{2} \text{NaClO}_3$ $\frac{1}{2} \text{Na}_2\text{Cr}_2\text{O}_7$	30	11.5 29.0 43.0	70.4 70.1 70.0	27.4 25.6 23.8	2.2 4.3 6.2	Hazard Hazard Hazard

with sodium chlorate. Admixture of small quantities of the dichromate salt with organic material does not affect the combustibility of the material appreciably, whereas a small amount of chlorate renders it hazardous.

The results show that, even under dry conditions, organic material contaminated with sodium dichromate can scarcely be considered dangerous. Mixtures containing high percentages of dichromate, however, may be somewhat dangerous. In order to ensure safety under all conditions, tests were made on mixtures composed of five-sixths sodium dichromate and one-sixth anhydrous calcium chloride. The results obtained for a 60% solution of this mixture are presented in Table XII. This mixture was found to be safe at a relative humidity of 29%, showing that when a small amount of calcium chloride is added to sodium dichromate this material is entirely safe.

Mixtures of sodium dichromate and sodium chlorate have been used as commercial herbicides, and although these may be effective from the standpoint of weed eradication, there is no reason to believe that the small amount of water held by the sodium dichromate will cause any significant reduction in the fire risk. Mixtures composed of equal parts of sodium dichromate and sodium chlorate, and two-thirds sodium dichromate and one-third sodium chlorate, were tested and the results are also given in Table XII. Both of these mixtures were, if anything, more hazardous than similar concentrations of pure sodium chlorate at relative humidities up to 43%, the highest value tested. Herbicides of this composition are therefore extremely dangerous.

Experiments with Potassium Permanganate

The effect of potassium permanganate, another oxidizing agent, on the combustibility of organic material was tested, although this chemical is not commonly used as a herbicide, and appears to be useless for this purpose (2). Test pieces of cloth and filter paper were soaked in an aqueous solution of potassium permanganate, saturated at 25° C. (7.6%). These pieces darkened considerably on drying but when conditioned at a relative humidity of 29% they were no more combustible than the untreated material. Apparently potassium permanganate is not hazardous, although it oxidizes the organic matter to some extent at room temperature.

Relative Humidity in Relation to Fire Risk

A survey of the results obtained shows that the fire risk involved in the use of a chlorate-containing herbicide will depend largely on the relative humidity to which material, contaminated with the herbicide, is exposed. To obtain some information on the relative humidity of the atmosphere, official records (4) were consulted.

The important point from the standpoint of this investigation was to determine how frequently the humidity fell below certain minimal values. This was done by obtaining the number of days on which the humidity fell below 30 and 40% at five western and one eastern station during the months

of June, July and August, for the five-year period 1924 to 1928 inclusive. At most of these stations the observations were made in the morning and evening only, when the temperature was usually lower than the maximum for the day, and consequently give humidity readings higher than the minimum values for most days. By assuming that the total water content of the air was the same at the hottest period of the day as it was when the humidity was measured it was possible to calculate the minimum relative humidity for any day. When this correction was applied to the data from the western stations it was found almost to double the number of days the humidity fell below 30%.

The summarized data are presented in Table XIII. This table is divided into three sections. The first section shows the number of days during the

TABLE XIII

NUMBER OF DAYS ON WHICH THE RELATIVE HUMIDITY FELL TO 30 AND 40%, OR LOWER, DURING JUNE, JULY AND AUGUST AT SELECTED STATIONS. DATA FOR YEARS 1924 TO 1928 INCLUSIVE

Year	Edmonton	Medicine Hat	Moose Jaw	Saskatoon	Winnipeg*	Ottawa
Number of days on which the observed humidity fell to 30% or lower						
1924	11	21	2	1	4	0
1925	14	26	6	0	1	0
1926	6	21	7	0	3	0
1927	3	4	1†	0	12†	0
1928	0	5	0	0†	0	0
Number of days on which the humidity probably fell to 30% or lower during hot part of day						
1924	19	36	3	11	Observations, as given above, were taken at 2 p.m. Hence no calculations made.	0
1925	20	40	17	7		0
1926	10	27	11	16		0
1927	4	7	7†	1		0
1928	0	7	0	1†		0
Number of days on which the observed humidity fell to 40% or lower						
1924	30	45	10	5	21	0
1925	34	49	31	1	15	0
1926	29	47	20	2	28	0
1927	18	21	11†	0	21†	0
1928	10	19	0	0†	1	0

*Observations at Winnipeg taken at 2 p.m. At all other stations observations were taken in the evening, 5.45 p.m. to 8.00 p.m., exact time varying with station and year.

†Observations for two months only.

‡Observations for one month only.

three-month period on which the observed humidity was below 30%; the second section shows the number of days on which the humidity was probably 30%, or lower, during the hot part of the day; the final section shows the number of days on which the observed humidity fell below 40%. The number of days on which the relative humidity fell below 40% during the period of maximum temperature was not calculated, but this does not affect the conclusions to be drawn, since the figures show that even the observed humidity is frequently below 40%.

The results show that low relative humidities are quite frequent in the drier regions of western Canada. Thus at Medicine Hat in 1925 there were 26 days on which the observed humidity fell to 30% or lower, 40 days on which the humidity was probably below 30% during the hot part of the day, and 49 days on which the observed humidity fell to 40% or lower. Low relative humidities are less frequent at the other western stations, but it is the extreme conditions that must be taken into account. In contrast to the above, the observed relative humidity at Ottawa never fell below 40%.

It is unlikely that the relative humidity remains below these low values for more than a few hours, and it is only during this period that a herbicide containing chlorate and sufficient water-absorbing chemical for protection at higher humidities will involve a fire risk. It was found in this investigation that most of the treated test material dried rather slowly when exposed to atmospheres of lower relative humidity, but gained weight quite rapidly when placed in atmospheres of higher relative humidity. Slow drying was in particular evidence when the moisture-retaining salt passed through a transition point. These observations support the contention that the period of danger may be of shorter duration than the period of low humidity. On the other hand it must be remembered that the humidity will normally decrease gradually, and it is possible that the herbicide will remain in practical equilibrium with it. In the interests of safety, therefore, it seems best to regard the herbicide as dangerous during the entire period that the humidity is below a certain value dependent on the kind and amount of the water-absorbing chemical present in the mixture.

As the relative humidity at western points frequently falls below 40%, it is evident that any chlorate herbicide in general use must contain sufficient protecting chemical to render it entirely safe at this relative humidity. To produce a chlorate herbicide that would be safe at relative humidities of 30% or less, would require a large proportion of protecting chemical and would doubtless prove to be impracticable owing to the cost and loss of herbicidal power. Considering this question of practicability, and bearing in mind the probability that this low relative humidity seldom occurs for more than short periods even in the drier districts, it would appear justifiable to employ a herbicide that, although not entirely free from risk, would be no more than a doubtful hazard under these conditions.

Conclusions

Accepting as safe herbicides only those that are not hazardous at 40% relative humidity, and no more than a doubtful hazard at 30% relative humidity, regardless of the proportions of herbicide and combustible material, only a few of the numerous mixtures tested come within this category. These are:* (1) one-half sodium chlorate plus one-half calcium chloride; (2) two-thirds sodium chlorate plus one-third magnesium chloride; (3) one-half sodium chlorate plus one-half sodium hydroxide; (4) two-thirds barium chlorate plus one-third calcium chloride; (5) two-thirds barium chlorate plus one-third magnesium chloride; (6) five-sixths sodium dichromate plus one-sixth calcium chloride; and (7) five-sixths sodium dichromate plus one-sixth magnesium chloride.

Comparing the first two mixtures listed, it seems probable that the superior protecting action of magnesium chloride over calcium chloride is exaggerated by these proportions. Calcium chloride does not provide the required protection when it forms only one-third of the mixture, as does magnesium chloride, but it would probably have been effective at some proportion between one-third and one-half of the mixture, had such a proportion been tested.

The value of chlorate-sodium hydroxide mixtures is somewhat doubtful. According to these tests this mixture is not hazardous, but exposure to air would convert much of the hydroxide to carbonate. Mixtures composed of one-half sodium chlorate plus one-half sodium carbonate were found to be hazardous, and although a mixture composed of one-half sodium hydroxide originally would correspond to a somewhat higher proportion of carbonate, it is doubtful, judging from the data in Table V, if such a mixture would meet the above requirements for a safe herbicide.

Barium chlorate has to be mixed with only one-half its weight of either anhydrous calcium chloride or magnesium chloride to be safe. Since calcium chlorate alone is less hazardous than barium chlorate alone it seems likely that these proportions of calcium and magnesium chlorides would also remove any fire risk involved in the use of calcium chlorate. Sodium dichromate is comparatively non-hazardous and is rendered entirely safe when either calcium or magnesium chloride forms one-sixth of the mixture.

The herbicidal power of these mixtures must be taken into account in considering which ones would be most valuable in practice. Sodium dichromate appears to be more toxic than sodium chlorate, when tested on annual weeds in a greenhouse (2), but the results of field experiments (10) indicate that it is less effective than sodium chlorate for eradicating perennials. Herbicides based on sodium dichromate are therefore of uncertain value at the present time.

Greenhouse tests of toxicity to annuals (2) indicate that sodium chlorate is more toxic than barium chlorate. This might be expected, since the per-

*Proportions of all chemicals are on an anhydrous basis.

centage of chlorate ion in sodium chlorate is higher than in barium chlorate. Some idea of the relative herbicidal power of the "safe" chlorate herbicides listed above can be obtained from the percentage of chlorate ion they contain. This information is shown in Table XIV, the chlorate being expressed as a percentage of the hydrated material, since this is the form that will be used in practice. The composition of sodium chlorate is also shown for comparison.

Judging from the chlorate content alone the mixture composed of two-thirds sodium chlorate and one-third magnesium chloride would be the most effective of the safe herbicides. However, it would only be about one-half as toxic as pure sodium chlorate. The other three mixtures have about the same chlorate content and would only be about

one-third as toxic as sodium chlorate. These deductions do not take the herbicidal power of the water-absorbing chemical into account. Preliminary tests (2) indicate that the minimum lethal dose of calcium chloride is about twice that of sodium chlorate. If magnesium chloride has about the same toxicity it is doubtful if these added chemicals contribute much to the killing power of the herbicide.

TABLE XIV
CHLORATE CONTENT OF "SAFE" HERBICIDES

Composition of mixture on anhydrous basis	Chlorate in final hydrated mixture, %
$\frac{2}{3} \text{NaClO}_3 + \frac{1}{3} \text{CaCl}_2$	26.3
$\frac{2}{3} \text{NaClO}_3 + \frac{1}{3} \text{MgCl}_2$	37.9
$\frac{2}{3} \text{Ba}(\text{ClO}_3)_2 + \frac{1}{3} \text{CaCl}_2$	26.8
$\frac{2}{3} \text{Ba}(\text{ClO}_3)_2 + \frac{1}{3} \text{MgCl}_2$	25.8
NaClO_3	78.4

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Addendum

Since the submission of this paper for publication Tingey (13) has reported spontaneous combustion of weeds on plots sprayed two hours previously with Atlacide. Thermohygrograph records show that, about the time combustion took place, the atmospheric temperature rose sharply from 65 to 75° F. and the relative humidity fell from 66 to 31%. Atlacide, however, is not calcium chlorate as stated by Tingey, but a mixture of approximately two-thirds sodium chlorate and one-third calcium chloride. The results presented in Table III of the present paper show that organic matter mixed with material having the composition of Atlacide is somewhat dangerous at relative humidities below 53%, and will become a distinct fire hazard at the low humidity reported by Tingey. Atlacide should not be confused with pure calcium chlorate; the results shown in Table X of the present paper were obtained with the latter chemical.

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STUDIES ON FOOT AND ROOT ROT OF WHEAT

II. CULTURAL RELATIONSHIPS ON SOLID MEDIA OF CERTAIN MICRO-ORGANISMS IN ASSOCIATION WITH *Ophiobolus graminis* SACC.¹

By W. C. BROADFOOT²

Abstract

The antagonistic and compatible growth relationships of 66 cultures of bacteria and fungi, most of which were from the soil, towards *O. graminis* on potato dextrose agar and Molisch's salt peptone agar, were compared with the effect of each on the virulence of this pathogene on wheat seedlings in open soil culture.

Of the 21 cultures which controlled the virulence of *O. graminis* in the soil, only 15 of these were antagonistic on potato dextrose agar, while of the 45 cultures which gave intermediate or no control, 17 were compatible and 28 were decidedly antagonistic. From data secured indirectly, the antagonism or compatibility of the micro-organisms toward *O. graminis*, observed on potato dextrose agar, did not seem to depend on active alkali or acid more than on other metabolic products. The study apparently demonstrates that the growth reaction of various micro-organisms and *O. graminis*, associated on the two solid media used, is not a reliable indication that the same micro-organism will or will not suppress the virulence of this pathogene on wheat in soil in open pot culture.

Ever since pure-culture methods have been perfected, the usual procedure in investigations has been with one organism kept pure and free from contamination. However, in nature, and especially in the case of soil-borne diseases, it would seem that more than one micro-organism is frequently associated in the host-parasite relationship.

Porter (15), Machacek (13), and later Fawcett (10) have emphasized the association effects of other micro-organisms in the production and symptoms of certain diseases in plant tissue. Savastano and Fawcett (20), Vasudeva (23, 24), Bamberg (1), Sanford and Broadfoot (18), Henry (11, 12), Moritz (14), Endo (8, 9), Broadfoot (2), and others have dealt with cases in which certain other fungi and bacteria modified the virulence of plant diseases. Dickson (6), Vanterpool (22), Valleau and Johnson (21), and others have demonstrated that the symptoms characteristic of a single virus may be altered by mixing certain plant viruses. Fawcett (10) mentions two general types of investigations in this connection; *viz.*, (1) the study in a quantitative as well as qualitative way of the effect of known mixtures or combinations of micro-organisms in culture media, and (2) the study in the same way of the effect on development of diseases by inoculation of plants with known mixtures of micro-organisms.

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In the work of Sanford and Broadfoot (18), which deals with the second type of investigation, the virulence of *Ophiobolus graminis* on wheat plants was profoundly modified, and at times controlled, by the association of cultures or filtrates of a number of soil-inhabiting bacteria and fungi. In producing this condition, there did not appear to be any difference whether fungi or bacteria, as classes, were concerned, but rather it was a case of the kind of organism or organisms which were associated. That the suppressive effect was probably due, directly or indirectly, to toxicity or staling products of the living cultures, or of their filtrates, and not to competition or to the hydrogen ion reaction was strongly suggested.

As a result of these conclusions it was decided to culture each of the micro-organisms, used in the original experiment mentioned, with *O. graminis* on a solid substrate, to see if the growth effects would be the same as in the soil. Should there be uniformity in this regard, a convenient and rapid method would be available for observing the probable antagonism or compatibility of such associations in the soil.

Material and Methods

The suitability of the following media for culturing *O. graminis* was tested: Dox's inorganic salt; Brown's synthetic; Czapek's; Harvey's glucose-glycerol; Molisch's salt peptone; Peptone sucrose; Waksman's sucrose peptone; Bezancon's glycerol peptone; Zike's magnesium sucrose asparagin; Woltze's sucrose ammonium sulphate; Heinemann's asparagin; Bokorny's ethyl alcohol ammonium sulphate; and potato dextrose. As *O. graminis* grew best on Molisch's salt peptone agar and on potato dextrose agar, these were employed.

A uniform portion of inoculum of *O. graminis* was planted on potato dextrose agar approximately 30 mm. from the edge of the Petri plates, and allowed to grow for three days, then the other organism was placed about 30 mm. from the growing colony of *O. graminis*. Separate tests were made of each of the micro-organisms studied. Each test was in triplicate. The general experiment was repeated four times. The first and second times, the cultures were grown on 15 cc. of potato dextrose agar, but the third and fourth times, on 25 cc. of potato dextrose agar and Molisch's salt peptone agar, respectively, contained in 250-cc. Erlenmeyer flasks. The initial pH reaction of the potato dextrose agar was 6.7, and that of Molisch's salt peptone 5.7. Notes were taken on the degree of antagonism or compatibility between *O. graminis* and the various introduced micro-organisms after the cultures had incubated 18 days.

Preliminary study in 1930 suggested that the growth reaction could be divided for the purpose intended into two main types, *viz.*, antagonistic and compatible. The former type is indicated by definite antagonism between the cultures, and compatibility by an intermingling or growing together of the two organisms. A subdivision of each general type was made as follows:

I. Antagonistic

- A. A distinct neutral zone between *O. graminis* and the introduced organism.
- B. A distinct line of demarcation between *O. graminis* and the introduced organism.

II. Compatible

- A. *O. graminis* grows over introduced organism.
- B. Introduced organism grows over *O. graminis*.

The two classes, indicating the two degrees of antagonism, are illustrated in Fig. 1, I (A) and I (B), respectively. The non-antagonistic or compatible types of growth are illustrated in Fig. 1, II (A) and II (B), respectively.

Zeller and Schmitz (26), Cook (4), Porter (16, 17), Brown (3), Machacek (13), Endo (7) and others have studied the association of phytopathogenes in culture. Endo (7) of Japan, classifying in plate culture the growth reaction between *Hypochnus centrifugus* and other micro-organisms, apparently used a similar scheme to that employed in this study, except that an extra type (class I (B)) has been described here. Two of his classes appear to be the same as ours, i.e., I (A) and II (B). Endo describes Class II (B), where the introduced organism grows over the other, as antagonistic; but we feel that it should be classed as compatible, since both organisms intermingle. Apparently he did not recognize, or at least describe, a type corresponding to Class I (B), where there is a sharp line of demarcation between the two organisms. His Fig. 2, in Plate III, illustrating the growth relation between *H. centrifugus* and *Bacillus cereus*, suggests the presence of a type of reaction similar to class I (B) used here.

Experimental Results

Association Effects on Solid Media

The growth reaction of each of 66 fungi and bacteria when associated with *O. graminis* on potato dextrose agar and on Molisch's salt peptone agar is given in Table I, according to the types indicated under methods. There was, except for minor variations, fairly good agreement in the results obtained in the three tests made on potato dextrose agar. In the case of Molisch's salt peptone agar, the growth reaction was frequently different from that on potato dextrose agar. This would be expected, since the same organism with its complement of enzymes might produce different end-products of metabolism on media of different composition. For example, 38 of the 66 micro-organisms mentioned above were antagonistic to *O. graminis* on both media, while nine of them were compatible. On the other hand, cultures Nos. 16, 23, and 49 were compatible on Molisch's salt peptone agar and antagonistic to *O. graminis* on potato dextrose agar. Again, cultures Nos. 18, 33, 38, 44, 48, 51, 61, and 63 were antagonistic on the former and compatible on the latter substrate.

TABLE I

THE GROWTH REACTION OF 66 CULTURES OF FUNGI AND BACTERIA IN ASSOCIATION WITH *Ophiobolus graminis* SACC. ON TWO NUTRIENT AGARS^a, AND THE EFFECT OF EACH CULTURE ON THE VIRULENCE OF THE PATHOGENE ON WHEAT SEEDLINGS IN SOIL CULTURE, AND ALSO THE pH VALUES OF THE POTATO DEXTROSE CULTURE DECOCTION

Culture	Potato dext.				Molisch's pept.				pH Pot. dext. cult. decoc'n°	Effect on virulence of pathogene ^d
	Growth type ^b				Growth type					
	I		II		I		II			
	A	B	A	B	A	B	A	B		
1. <i>Helminthosporium sativum</i>		X				X			6 7	NC
2. <i>Fusarium culmorum</i>				X				X	5 6	NC
3. <i>Wojnowicia graminis</i>		X			X				6 6	C
4. <i>Leptosphaeria herpotrichoides</i>		X				X			6 1	IC
5. <i>Sclerotinia</i> sp.	X					X			5 0	NC
6. <i>Fusarium culmorum</i>				X				X	6 0	IC
7. <i>Ascochyta graminicola</i>		X			X				6 7	NC
8. Bacteria	X				X				7 3	NC
9. Fungus				X				X	6 8	NC
10. <i>Plenodomus meliloti</i>		X			X				6 2	C
11. <i>Helminthosporium sativum</i>		X				X			7 0	C?
12. <i>Rhizoctonia</i> sp.				X				X	6 7	NC
13. <i>Actinomyces</i> sp.	X				X				6 3	IC
14. <i>Bacterium translucens undulosum</i>	X					X			8 4	C
16. Fungus		X					X		6 0	IC
17. Fungus		X				X			5 3	IC
18. <i>Typhula graminum</i>			X			X			6 7	IC
19. Bacteria	X				X				7 2	C
20. Bacteria	X				X				7 3	C
21. Bacteria	X				X				5 7	NC?
22. Bacteria	X				X				4 3	C
23. <i>Plenodomus destruens</i>		X					X		6 3	NC
24. <i>Plenodomus chelidani</i>		X				X			6 4	C
25. Fungus		X			X				7 0	NC
26. Fungus			X					X	6 4	C
27. Fungus		X			X				5 3	NC
28. <i>Actinomyces</i> sp.		X			X				7 3	NC
29. Bacteria	X					X			5 6	IC
30. Bacteria	X				X				7 2	C
31. Bacteria		X							5 8	NC
32. <i>Actinomyces</i> sp.		X			X				5 8	NC
33. Bacteria			X		X				5 2	NC
34. Bacteria	X								7 5	NC
35. Bacteria			X						7 3	IC
36. Bacteria	X				X				7 0	C
37. Bacteria	X				X				5 9	IC
38. Bacteria			X			X			6 3	C
39. Bacteria			X						6 5	IC
40. Bacteria			X						7 1	IC
41. Bacteria	X				X				7 1	C
42. Bacteria			X						7 1	C
43. Bacteria	X				X				7 6	C
44. Bacteria			X		X				6 8	NC
45. Bacteria		X			X				7 0	IC

^a Potato dextrose agar and Molisch's salt peptone agar.

^b Types I, antagonistic; II, compatible; see context for description of subtypes A and B.

^c pH values of potato dextrose culture decoction; and ^d effect on virulence of *O. graminis* (C, control; IC, intermediate control; NC, no control), data of both taken from Table II of context reference (18).

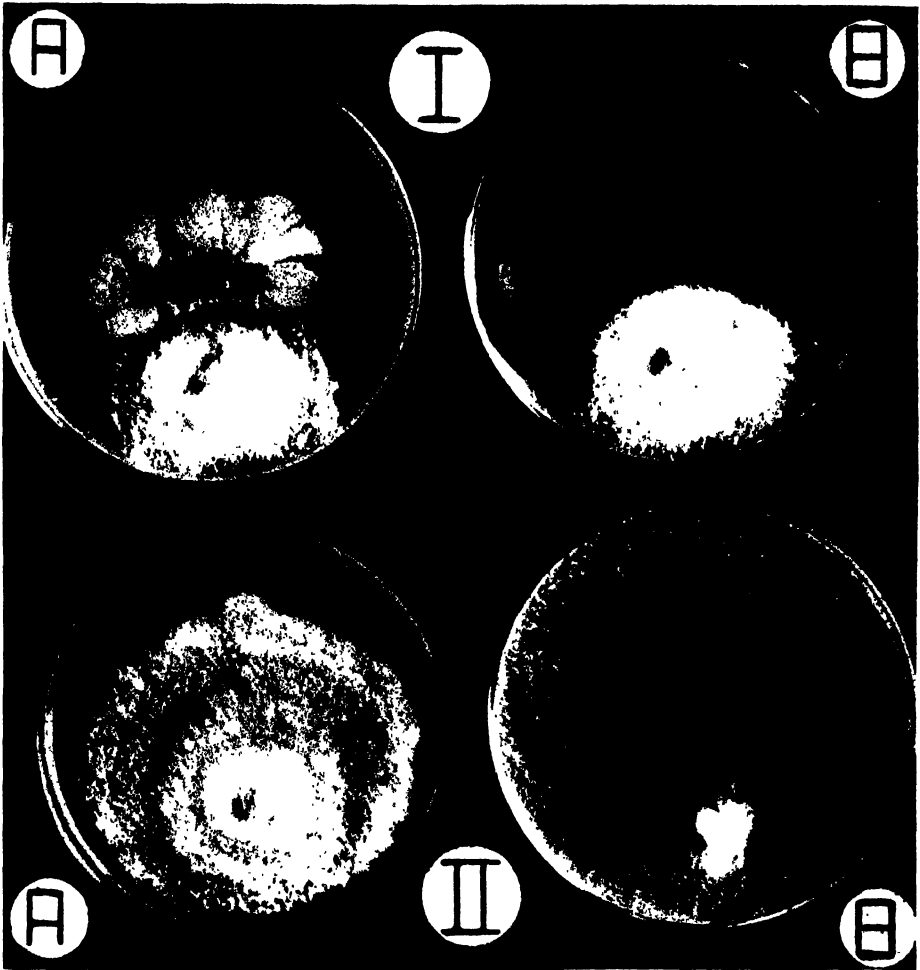


FIG. 1. Examples of antagonism and compatibility between *Ophiobolus graminis* Sacc. and other micro-organisms on potato dextrose agar. I. Antagonism. Subtype (A), a distinct neutral zone between *O. graminis* and culture No. 20. Subtype (B), a sharp line of demarcation between *O. graminis* and culture No. 1. II. Compatibility. Subtype (A), *O. graminis* grows over culture No. 55. Subtype (B), culture No. 12 grows over *O. graminis*.

TABLE I—Concluded

Culture	Potato dext.				Molisch's pept.				pH Pot. dext. cult. decoc'n ^a	Effect on virulence of pathogene ^d
	Growth type ^b				Growth type					
	I		II		I		II			
	A	B	A	B	A	B	A	B		
46. <i>Ophiobolus graminis</i>			X				X		6.5	NC
47. Bacteria	X				X				7.8	C
48. <i>Bacillus fecalis alcaligenes</i>			X		X				8.8	NC?
49. <i>Botrytis cinerea</i>		X					X		3.8	C
50. <i>Bacillus coli</i>			X						7.0	C?
51. <i>Bacillus butyricum</i>			X		X				7.5	NC
52. <i>Bacillus cinnebareus</i>		X			X				7.6	IC?
53. <i>Bacillus cereus</i>			X				X		8.4	C?
54. <i>Bacterium anthracoides</i>	X				X		X		8.5	IC?
55. <i>Bacillus mycoides</i>			X				X		6.2	NC
56. <i>Bacillus megaterium</i>	X				X				6.7	NC
57. <i>Bacillus subtilis</i>			X				X		9.0	C?
58. <i>Bacillus vulgatus</i>	X				X				6.7	C
59. <i>Bacillus ramosus</i>	X					X			6.7	NC
60. Bacteria			X						6.7	NC
61. Bacteria			X		X				7.6	NC
62. <i>Penicillium</i> sp.		X				X			5.5	IC?
63. <i>Rhizopus</i> sp.				X		X			3.8	NC
64. Bacteria		X				X			6.7	NC
65. Bacteria	X				X				7.3	NC
66. Bacteria	X				X				8.8	NC
67. Bacteria	X				X				6.7	NC

Association Effects on Potato Dextrose Agar Compared with those in the Soil on the Virulence of O. graminis on Wheat Seedlings

Taken from the original paper of Sanford and Broadfoot (18) and also included in Table I for comparison, are the association effects of the same cultures on the virulence of *O. graminis* on Marquis wheat seedlings grown in soil. One might expect that each of the organisms that controlled in soil culture the virulence of *O. graminis* on wheat seedlings, would also be antagonistic when grown with it on potato dextrose agar. However, this does not appear to be the case. Forty-three of these micro-organisms were antagonistic, and 23 were compatible. Of the 21 which controlled the virulence of *O. graminis* in soil culture (Nos. 3, 10, 11, 14, 19, 20, 22, 24, 26, 30, 36, 38, 41, 42, 43, 47, 49, 50, 53, 57, and 58), fifteen of these (Nos. 3, 10, 11, 14, 19, 20, 22, 24, 30, 36, 41, 43, 47, 49, and 58) were antagonistic, whereas six (Nos. 26, 38, 42, 50, 53, and 57) were not antagonistic.

On the other hand, it might be expected that the cultures which gave intermediate or no control of *O. graminis* in soil culture would not be antagonistic on potato dextrose agar. Of the 45 cultures which gave intermediate or no control of *O. graminis* in soil culture, 17 were not antagonistic, while 28 were decidedly antagonistic. The antagonistic cultures were Nos. 1, 4, 5, 7, 8, 13, 16, 17, 21, 23, 25, 27, 28, 29, 31, 32, 34, 37, 45, 52, 54, 56, 59, 62, 64, 65, 66, and 67.

Compatibility and Antagonism in Relation to Acids and Alkalies Produced in Culture

It was believed possible that the association effects observed on solid media might be influenced directly by acids or alkalies produced by the associated fungi. In the absence of information obtained directly from the solid media on which the micro-organism grew, it was decided to use, as a basis for comparison, the data published by Sanford and Broadfoot (18), which are listed in Table II. These values represent the final reaction of the potato dextrose decoction in which the various micro-organisms grew, and they should indicate the tendency of each culture to produce acids or alkalies on potato dextrose agar of essentially the same composition as the decoction.

Thus, in comparing the pH values of the decoction of the 66 cultures, and considering first the 43 cultures, which on the solid substrate were antagonistic to *O. graminis*, we find (Table II) that 11.6% of these had pH values between 3.8 and 5.4; 14.0% between 5.5 and 5.9; 58.1% between 6.0 and 7.4; 9.3% between 7.5 and 7.9; and the remaining 7.0%, values between 8.0 and 9.0. Of the 23 compatible cultures, 8.7% had values between 3.8 and 5.4; 4.3% between 5.5 and 5.9; 65.2% between 6.0 and 7.4; 8.7% between 7.5 and 7.9; and the remaining 13.0% of the cultures, values between 8.0 and 9.0. Apparently 58.1% of the antagonistic cultures, and 65.2% of the compatible cultures had pH values between 6.0 and 7.4, which range is, according to Davis (5), and Webb and Fellows (25), the optimum for good growth of *O. graminis* although depending somewhat on the strain concerned, as well as on the physical and chemical nature of the media.

Thus, on the basis of comparison used above, the antagonism or compatibility exhibited was, in many cases, not a direct result of active acidity or alkalinity, but rather due to other metabolic products of one or both associated micro-organisms, which conclusion agrees with that of Machacek (13).

TABLE II

THE ANTAGONISTIC OR COMPATIBLE GROWTH TYPES OF 66 MICRO-ORGANISMS IN ASSOCIATION WITH *Ophiobolus graminis* SACC. ON POTATO DEXTROSE AGAR ARRANGED ACCORDING TO THE pH VALUES OF THE POTATO DEXTROSE CULTURE DECOCTION IN WHICH THEY WERE ALSO CULTURED

Growth types	pH value classes*										Total no.
	3.8-5.4		5.5-5.9		6.0-7.4.		7.5-7.9		8.0-9.0		
	No.	%	No.	%	No.	%	No.	%	No.	%	
Antagonistic Compatible	5	11.6	6	14.0	25	58.1	4	9.3	3	7.0	43
	2	8.7	1	4.3	15	65.2	2	8.7	3	13.0	23
	7	10.6	7	10.6	40	60.6	6	9.1	6	9.1	66

*Data taken from Table II, context reference (18).

Discussion

Considering the results obtained from the foregoing studies, many of the micro-organisms which exercised a marked degree of control on the virulence of *O. graminis* on wheat seedlings in soil, in open pot culture, were not antagonistic to the pathogene on potato dextrose agar and on Molisch's salt peptone agar. In fact, more than one-half of the 66 cultures of fungi and bacteria employed in the study, which exhibited little or no effect on the virulence of *O. graminis* in soil, were decidedly antagonistic on potato dextrose agar. Likewise, many that controlled or greatly suppressed the virulence of this pathogene in soil were compatible on the media mentioned. Also many of the cultures which were compatible on potato dextrose agar were antagonistic on Molisch's salt peptone agar, and *vice versa*. A possible explanation for this, as it concerns both artificial media and the soil, is that the various micro-organisms, each with its complement of enzymes, produce substances which differ in kind and amount, according to the substrate.

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AN EXAMINATION OF IMMUNE SERUM LIPOIDS FOR THE COMPLEMENT FIXING ANTIBODIES OF TUBERCULOSIS¹

BY A. HAMBLETON²

Abstract

Lipoids which were extracted from immune tuberculosis serum at low temperatures by various procedures and solvents were examined by the tuberculo-complement-fixation test; antibodies were not found in the lipid fractions, but were retained with the protein fraction of the serum. The use of desorbents, benzoic acid in alcohol, or saponin,—during the extraction, or the preliminary destruction of serum proteins by trypsin, did not result in antibodies being detected in the lipid fractions. These results conform with other evidence which suggests that antibodies are protein in nature.

There exist at present no adequate data concerning the chemical and physical structure of antibodies. Much of the best work available is in the main an attempt to determine whether the antibodies are proteins, or are substances of unknown constitution which are firmly attached to the proteins. In some respects antibodies have properties similar to the enzymes, and while the structure of enzymes is still unknown, it is noteworthy that two enzymes, urease (19) and pepsin (14), and the enzyme-like substance insulin (6), have been prepared in a crystalline form, and are essentially protein in nature.

The present tendency is to regard antibodies as modified proteins, and the evidence in favor of this protein nature of antibodies has been reviewed by Strumia and coworkers (18), Heidelberger (7) and Wells (21). Locke and Hirsch (10) stated in 1925, "The general belief in the protein containing character of the antibodies is founded on the experimental fact that they may be precipitated with definite protein fractions of the immune serum, and on the theoretical implication that the proteins, alone of the constituents of animal tissue, have the requisite versatility of chemical and spatial configuration to account for the specificity and multiplicity of immunologic reactions". At the present time it is doubtful if either of the two points raised by Locke and Hirsch can be considered valid. If immune-serum proteins are precipitated by different methods, the antibodies do not always follow the same protein fraction. For example, when precipitated by ammonium sulphate, tuberculosis antibodies are found to be practically equally divided between the euglobulin and pseudoglobulin fractions (12, 13). But if euglobulin is precipitated by CO₂ from diluted immune serum, the euglobulin contains little or none of the antibody (8). In fact, there has been so much contradictory evidence as to which protein fractions contain a given antibody, that Mudd (12) refers to it as "the perennial question of the distribution of antibodies amongst serum proteins". Again, recent work on the specific

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polysaccharides of various bacteria by Heidelberger, Avery, Goebel and others (7) indicates that the polysaccharides offer a range of combinations adequate to account for any specific immune properties.

It is possible that many solutions of antibodies have been reported protein-free merely because the chemical tests for proteins are not sufficiently sensitive. On the other hand, antibodies of several types have been purified by Olitzki and Fränkel (15) by means of adsorption on kaolin with subsequent elution, and neither chemical tests nor the exceedingly delicate anaphylactic tests with guinea pigs indicate the presence of protein. The hemolytic antibodies purified by Uchida (20) by specific adsorption on red cells also fail to produce anaphylaxis, although the highly purified hemolytic antibody solutions of Locke and Hirsch (10) produced anaphylaxis when used in sufficiently large quantities. Again, if antibodies are "appropriately modified serum proteins", as Heidelberger (7) suggests, it is strange that they should be unattacked by trypsin under conditions which destroy the remainder of serum proteins. This resistance to trypsin was pointed out by Huntoon (9) in the case of pneumococcus antibodies, by Uchida (20) for hemolytic antibodies, and by Pinner (16) for tuberculosis antibodies.

Since the evidence in favor of the protein nature of tuberculosis antibodies is far from conclusive, it appeared desirable to make an examination of immune-serum lipoids, to find if the complement fixing antibodies can under any circumstances be found in this lipid fraction. Should this be the case, it would make available a simple method of preparing on a large scale antibody solutions which would be almost completely free from protein. This would facilitate the extensive treatment of experimental animals with the antibody preparations, without danger of anaphylactic reactions, and so assist in appraising the clinical value of tuberculosis antibodies.

No adequate data were found in the literature to indicate that the tuberculosis antibodies were of lipoidal nature. Hodge and Maclellan (8) found that when immune human serum was dried on filter paper and extracted with a mixture of alcohol, ether and chloroform, the serum lost its complement fixing power. Their results do not prove whether they had extracted the antibodies into the solvent, or had destroyed them, since the lipid extracts obtained by this method were so highly anticomplementary that they could not be tested by the complement fixation reaction. It is probable that the method employed had simply destroyed the antibodies, as later work has shown that they can be destroyed by ether (16).

Pinner, in the trypsin digestion experiments to which reference has been made, noted that when toluene was used as an antiseptic the antibodies disappeared from the aqueous solution; he suggested that possibly the antibodies had dissolved into the toluene layer, although his report does not mention any direct examination of the toluene layer for antibodies.

It was obvious from the literature that much of the work concerning the antibodies of tuberculosis had been carried out with serum fractions which, while nominally protein, would also from their method of preparation contain

serum lipoids. For example, Strumia and coworkers (18) have used globulin fractions obtained from immune serum by ammonium sulphate precipitation, although as pointed out by Pinner (16) the lipoids are carried down with the globulin precipitate.

An attempt at the direct extraction of tuberculosis antibodies by means of lipid solvents is reported by Nishida and Petroff (13). Samples of immune sheep serum were shaken with various solvents, and then held at 37.5° C. for 24 hr. The lipoids so extracted were emulsified in saline, and tested for their complement fixing properties. The authors concluded from their results that the complement fixing antibodies are not lipoids.

Experimental

In order to determine by extraction methods whether the antibodies are of lipoidal nature, three main types of experiment are necessary.

1. *Direct extraction.* This should succeed if the antibodies are in a free state in the serum, and if a suitable solvent and extraction procedure are employed.

2. *Desorption methods.* Should direct extraction fail, it is possible that the antibodies, although normally soluble in the organic solvent employed, are too firmly adsorbed by the serum protein. In this case it should be possible to displace the antibody from the protein by means of some other substance which is still more strongly adsorbed.

3. *Extraction after destruction of the serum protein.* Finally, there is the possibility that the antibodies, although of lipid nature, are chemically united to the serum protein. In this case, the antibodies would probably be set free after destruction of the serum protein by prolonged trypsin digestion, and should then dissolve in an appropriate lipid solvent.

The following is an outline of experiments which the writer has carried out by each of these methods.

DIRECT EXTRACTION

Preliminary experiments were carried out in this laboratory by P. M. Andrus, who duplicated and confirmed the negative findings of Nishida and Petroff to which reference has been made. By quantitative estimation of the lipoids so extracted, Andrus noted that this method was unsatisfactory in that the extraction of lipoids was far from complete; the prolonged contact of the fat solvents with the immune serum—24 hr.—also appeared undesirable. It was obvious that no conclusive results could be obtained without the use of more effective extraction methods. Taking cholesterol and total fatty acids as typical lipoids, various extraction methods were tried, of which the Soxhlet method was found to be the most satisfactory, provided that a suitable inert base was employed. Finely ground sodium sulphate was found to be the most satisfactory inert base, although sodium chloride, powdered

glass, filter paper, and sand can be used. Although plaster is widely used as an inert base, that used by the writer (Baker's c.p. product) gave very poor yields of lipid during the relatively short extraction periods employed.

Since the thermal death point of the complement fixing antibodies of tuberculosis is about 70° C., only those organic solvents which boil below this temperature could be employed in the ordinary Soxhlet extractor. To increase the range of solvents which could be used, a modified form of the apparatus was constructed which operated under reduced air pressure. This made it possible to adjust the boiling temperatures of a wide variety of solvents to any desired figure by adjusting the degree of vacuum. This vacuum type of extractor is described elsewhere (3).

Table I indicates the efficiency of this Soxhlet apparatus, when operating at reduced temperatures, in extracting fatty acids from sheep serum.

The usual technique for examining serum lipoids was as follows. Immune serum was prepared by intravenous injection of increasing doses of living human-type tubercle bacilli into a sheep. One cubic centimetre of the immune serum, either fresh or inactivated, diluted with 1 cc. of water, was well ground into 30 gm. of anhydrous sodium sulphate (Merck's Blue label). Plaster of Paris was used in place of sodium sulphate in some of the experiments, although as stated above it does not give complete removal of the lipoids. The mixture of serum and inert base was transferred to a large culture tube, covered by a plug of cotton wool, and connected to a high vacuum pump. The lower end of the tube was immersed in a water bath at 50° C. After $\frac{1}{2}$ hr., the contents of the tube was sufficiently dry to be removed and finely ground in a mortar, after which the powder was returned to the tube and dried for an hour longer under high vacuum at 50° C., during which time the tube was well shaken at intervals.

TABLE I
EFFICIENCY OF LOW TEMPERATURE SOXHLET EXTRACTIONS

Inert base	Solvent	Time and temperature of extraction	Fatty acid extracted, mgm. per 100 cc.	Efficiency of the extraction, %
Na ₂ SO ₄	Absolute ethyl alcohol	40 min. at 40° C.	103.4	99.3
Filter paper	Chloroform	40 min. at 40° C.	102.5	98.4
Plaster	Absolute ethyl alcohol	40 min. at 40° C.	73.5	70.5

NOTE.—The sheep serum used contained 104.2 mgm. of total fatty acid per 100 cc. by Bloor's method (2).

The dried powder was placed in a paper extraction thimble, and extracted in the Soxhlet extractor to which reference has been made. Ethyl alcohol, both absolute and 95%, methyl alcohol, acetone and chloroform were the

solvents most frequently employed, although methyl acetate, benzol and petroleum ether were also used. Each sample was extracted three times in succession, using fresh solvent each time, with the aim of completely removing the soluble fraction without, however, exposing to prolonged heating the material which was first dissolved. Each of the extraction periods was from 15 to 45 min., while the degree of vacuum was adjusted to cause the solvents to boil at various temperatures from 25° to 50° C.

Each extract was separately placed in a well-cleaned evaporating basin, and placed inside a vacuum desiccator, the latter being inside an incubator at 50° C. When the solvent was completely removed, the evaporating basin was wrapped in paper and stored in the ice box until the tuberculo-complement fixation test was carried out. Immediately prior to the test the sample was emulsified into buffered saline by rubbing with an agate pestle while slowly adding the saline. This gives satisfactory emulsions for extracts obtained by acetone or alcohol. Chloroform extracts usually were redissolved in that solvent, and slowly run into distilled water which was rapidly stirred by a mechanical stirrer. The chloroform was then removed by boiling under high vacuum, and the watery emulsion of lipoids made isotonic with sodium chloride. In the later work, this method of emulsification was used with all extracts, irrespective of the solvent employed for the extraction.

The technique used for the tuberculo-complement fixation test (hereinafter designated as T.C.F. test) was similar to the Petroff technique as detailed by Woolley (22, pp. 160-180) but additional tubes were used containing 2, 2½, 3, 4, 6 and 8 units of complement. The lipid emulsion which was tested in each tube corresponded to 0.1 cc. of the original immune serum.

Using this technique in the writer's earliest experiments a number of extracts were obtained which gave positive reactions in the T.C.F. test. About 25% of the extracts were too anticomplementary to give useful results. On extending the work by the use of other sheep whose serum had a very much higher titre of antibodies, using immune rabbit and guinea pig serum, and the greatest precautions in technique, it was found impossible to duplicate these early apparently positive reactions. The following are some points of technique which from later experience are believed to have contributed to the apparently positive results obtained at the outset of this work.

1. Where plaster was used as inert base, traces of this carried over into the extract can contribute to non-specific reactions, as shown in recent contributions from these laboratories (4, 5).

2. Emulsifying the lipid fraction by rubbing up from a porcelain basin is undesirable, as it was observed that the porcelain enamel was easily damaged and that the final emulsion contained fine porcelain particles; this porcelain emulsion alone gives an anticomplementary effect.

3. In the later work the T.C.F. test was modified. Two units of complement were used in each tube, and serial dilutions of the lipid emulsion were tested. By this dilution method extraneous non-specific factors are minimized.

Extensive work confirmed the conclusion that no true complement fixing antibody was dissolved out by any of the solvents employed. In addition to trying wide ranges of time and temperature for the extraction, the serum was in some experiments adjusted to various pH values from 5 to 9 before drying on the inert base, but in none of these extracts could complement fixing antibodies be detected. Quantitative estimation of the antibody in the extracted protein material remaining in the extraction thimble was attempted in almost all experiments in which sodium sulphate was used as inert base. After drying to remove the organic solvent, sufficient water was added to form at 35° C. a saturated sodium sulphate solution, which was filtered in the incubator. Of the precipitated protein, part was soluble in saline, but most of it was insoluble, due to being denatured, when acetone or alcohol was used as solvent. This insoluble fraction was brought into solution by digestion with a trace of trypsin, and the two protein fractions were separately tested for antibodies. Of 37 of these residual protein samples which were tested, 2 gave positive tests, 3 gave doubtful positive tests, 14 gave negative tests and 18 gave anticomplementary tests. These results give no definite information, as half of the samples are anticomplementary. Quantitative results on the extracted residues were satisfactory only when filter paper or clean sand was used as the inert base, and some solvent such as chloroform was employed, which does not denature the serum protein. Details of this are given below.

Efficiency of Methods Employed

The following trials were carried out to determine whether the methods employed give efficient extraction of lipoids and do not in themselves destroy the antibodies of tuberculosis.

1. *Effect of drying.* (a) Serum dried at 50° C. in vacuum on sodium sulphate is partly denatured, and gave inconsistent results when the recovered serum was tested for antibodies. Serum mixed in the cold with sodium sulphate so as to form a solid mass of $\text{Na}_2\text{SO}_4 \cdot 10 \text{ H}_2\text{O}$ crystals, can be recovered with but little loss of the antibodies. Nevertheless, alcohol, acetone and chloroform extracts of serum dried in this manner on sodium sulphate were always negative.

(b) Portions of immune sheep serum were dried on filter paper and on purified sand, while other portions were spread over the bottom of Petri dishes without mixing with any inert base. These samples were dried under various conditions, both exposed to the air and in high vacuum, at various temperatures from 37° to 45° C. In all cases the result was the same, namely, that on redissolving the dried serum, 50% of the original antibody titre remained. Hodge and Maclellan (8) obtained similar results with immune human serum dried on filter paper one hour at 45° C. in air.

2. *Effect of solvent.* Serum dried as above on filter paper or sand was covered with chloroform and allowed to stand one hour at room temperature. The chloroform was then allowed to evaporate, and the serum was recovered

by repeated extraction with saline. Results showed that the treatment with chloroform caused no destruction of antibodies. Similar trials with alcohol or acetone were not carried out, since they denature the serum protein.

3. *Efficiency of extraction methods.* As regards the percentage of total fatty acids extracted, details are given in Table I, and since in examining for antibodies three successive extractions were given, instead of only the one extraction used for the data in this table, it is obvious that the lipid recovery was very complete.

4. *Recovery of antibodies in protein residue after the extraction.* Immune serum, dried as above on filter paper or sand, was extracted at 40° C. for 30 min. with chloroform. The protein residue, after evaporating off the chloroform, was recovered by washing with saline, and showed no loss of antibodies. The chloroform extracts were all negative, as also were extracts in which acetone and alcohol were the solvents. With the last two solvents the antibody titre of the extracted protein could not be determined, as the proteins were denatured. These results are given in Table II, and indicate that all the antibodies, except those lost during the process of drying the serum, remained with the protein fraction.

Other Extraction Methods

Direct extraction methods other than those outlined above were also tried, of which only two examples need be quoted.

TABLE II
EFFECT OF DRYING AND OF ORGANIC SOLVENTS UPON IMMUNE TUBERCULOSIS SERUM

	Titre of antibodies in protein residue, units per cc.	Titre of antibodies in chloroform, acetone or alcohol extract, units per cc.
Serum dried on filter paper, 30 min. at 45° C. in air, then redissolved in saline without extraction by organic solvent	40	—
Serum dried on filter paper as above, then extracted 30 min. at 40° C. with chloroform	40	0
Serum dried on purified sand 1 hr. at 40° C. in air, then redissolved in saline	40	—
Serum dried on purified sand as above, then extracted 30 min. at 40° C. with chloroform	40	0
Serum dried on Na ₂ SO ₄ , then extracted with acetone, 30 min. at 40° C.	Protein denatured	0
Serum dried on Na ₂ SO ₄ , then extracted with absolute alcohol, 30 min. at 40° C.	Protein denatured	0

NOTE.—The titre of the fresh serum was 80 units per cc.

(a) Immune serum was well stirred in a mortar and alcohol or acetone slowly run in, so that the protein was precipitated in a finely divided form. This precipitate was further washed with fresh alcohol or acetone, and allowed to stand in one of these liquids for two hours at various temperatures from 20° to 40° C., the mixture being frequently shaken. In other experiments the precipitated protein was placed in an extraction thimble, and extracted under the reflux condenser of the vacuum extraction apparatus used in the previous experiments. These are not efficient extraction methods, since in all cases only about 80% of the total fatty acids are dissolved out by the solvents. No complement fixing antibodies were found in these acetone and alcohol extracts.

(b) Serum was rapidly stirred by a mechanical stirrer, while chloroform was slowly added, so that a thick white emulsion was formed. After standing for five days at 8° C. the emulsion was cracked by high speed centrifugation. No antibodies were found in the chloroform layer, while the separated serum retained its original titre of antibodies. Only 29% of the total fatty acids had been dissolved out of the serum by the chloroform.

DESORPTION EXPERIMENTS

Since direct extraction with lipid solvents failed to dissolve out the complement fixing antibodies, the following attempts were made to displace antibodies from the serum protein by means of other highly adsorbed substances, benzoic acid and saponin being employed for this purpose. In the recovery of insulin adsorbed upon charcoal, Moloney and Findlay (11) found that benzoic acid in alcoholic solution was the most effective of all the materials they tried for effecting this desorption. Saponin has been also used in experiments of this nature (1, 17).

To reduce the acidity of benzoic acid to that which the antibodies of tuberculosis would safely stand (pH 5.5 to 6.0) aqueous solutions of benzoic acid or mixtures of benzoic acid and sodium benzoate were mixed with immune serum so as to give in several samples a pH range of 5 to 7. Alcohol was then added in excess to completely precipitate the protein. After standing overnight at -10° C. the samples were centrifugated, and the protein residue was stirred for two hours at room temperature with a fresh solution of 50% alcohol containing benzoic acid—sodium benzoate mixture of the same pH as the first supernatant liquid. Both the first and second extracts were neutralized, the alcohol boiled off in a vacuum, and the samples were made isotonic for the T.C.F. test. Control tests showed that the concentration of benzoate employed did not interfere with the serology.

Somewhat similar experiments were made in which saponin was used. To 1 cc. portions of immune serum was added 0.5 cc. of 6% saponin, and then serial amounts of acetic acid were added to the tubes, giving a pH range from 7.6 to 5.2. Alcohol was then added to give a final concentration of 60%, and the samples were kept at -10° C. overnight. The samples were then held at 20° C. for one hour, during which they were frequently shaken, neutralized, concentrated to a small volume and slowly run into a large

volume of acetone at -10° C. to precipitate the saponin. Water was then added to the supernatant liquid, the acetone boiled off in a vacuum, and the samples made isotonic for the T.C.F. test.

Neither of the desorption methods outlined above yielded extracts containing complement fixing antibodies.

EXTRACTION FOLLOWING TRYPSIN DIGESTION

Finally, there remained the possibility that the antibodies were chemically united to the protein, and hence, even if of lipoidal nature, could not dissolve in the organic solvent. To examine this possibility, two experiments were made after trypsin digestion.

(a) A sample of immune-serum globulins, precipitated by ammonium sulphate, was subjected to simultaneous trypsin digestion and dialysis for 48 hr. at 47° C. Most, but not all, of the protein was destroyed, without destruction of the complement fixing power. This material was then dried on purified filter paper, and extracted 30 min. at 40° C. with chloroform in the vacuum extraction apparatus. This extract was completely negative in the T.C.F. test, while the residue on the filter paper, after drying off the chloroform and washing in saline, retained its complement fixing power.

(b) Immune-serum globulins were simultaneously digested with trypsin and dialyzed in flowing water for five days at 37° C.; and the resulting solution retained the antibodies. This solution was practically protein-free since it gave no precipitate on boiling after addition of sodium chloride and acetic acid; sulphosalicylic acid gave no immediate precipitate, although a slight sediment settled out after standing overnight. The digested sample was dried on purified plaster of Paris, and extracted in the vacuum type of Soxhlet apparatus with 95% alcohol for 30 min. at 45° C. The extract contained no complement fixing antibodies. Pinner (16) had noticed that if immune serum is covered with toluol while undergoing trypsin digestion, the antibodies disappear from the water phase. He tentatively suggested that the antibodies might have dissolved into the toluol layer. However, in attempting to use a wide variety of antiseptics (borate, salicylate, benzoate, chloroform, toluol, thymol, camphor, phenol) to prevent contamination of a trypsin digest of immune tuberculosis serum, the writer has always found that the antibody was destroyed by the joint action of trypsin and antiseptic. Pinner's result is apparently due to destruction of antibody by toluol plus trypsin, and not an indication that the antibodies are soluble in the toluol.

Discussion

The results of this work confirm the viewpoint that the antibodies of tuberculosis are not of lipoidal nature, since all the solvents and extraction methods tried, even after trypsin digestion of the serum protein, gave negative results. Further study of the chemical nature of these antibodies should apparently be directed either to those proteins which are resistant to trypsin, or possibly to the polysaccharides.

It is probable that the resistance of antibodies in general to trypsin digestion is related to the fact that many bacteria secrete proteoclastic enzymes. The function of an antibody apparently is to unite with its homologous organism, and in so doing modify its surface properties so as to facilitate humoral or cellular bacteriolysis. Obviously, any antibody which could be destroyed by its homologous micro-organism would be very ineffective.

Acknowledgments

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VIBRATIONS IN SOLID RODS AND DISKS¹

BY GEO. S. FIELD²

Abstract

I. A method is described for producing on the screen of a cathode ray oscillograph a curve showing the oscillations occurring in a solid rod or disk as the result of impact. These curves are usually quite complex, as they represent combinations of several different modes of vibration. They may be analyzed, however, so that for any specimen the various component frequencies may be determined. This has been done for a great number of rods and disks, and the experimental frequencies so obtained have been compared with those calculated from theory. A discussion of the results is given.

II. The solutions given by Love (5, p. 291) for the general equations of vibration applicable to transverse waves in cylindrical rods are examined, and it is shown that they do not apply to comparatively short rods. Other solutions of the same simple form are considered, and the conclusion is finally reached that a much more complex solution will be required to satisfy all the boundary conditions.

PART I

Introduction

The propagation of ultrasonic vibrations in solid rods has recently attracted considerable attention, and both theoretical and experimental work have been done to show the influence of frequency on the phase velocity of the longitudinal and flexural vibrations. The experimental method of analysis usually employed involves setting the rod into mechanical vibration by means of magnetostrictive or piezo-electric coupling with an electric oscillator. The resonant frequencies of the rod are found, the wave-length determined and a calculation then made of the wave velocity (1, 2, 6, 7, 8).

Recently a somewhat different method of studying vibrations in rods was devised, which shows in a quantitative way the numerous vibrations which may occur at the same time in a solid rod, and a short account of the technique was published (3). In this paper the apparatus is described more fully, and the results obtained so far are given and discussed.

Experimental

Utilizing the apparatus shown schematically in Fig. 1, the rod being studied is tapped at one end with a small hammer ten times a second. The resulting damped wave-trains in the solid are picked up by a small piece of piezo-electric quartz, cemented to one end. The slight e.m.f. appearing on the crystal is amplified by a six-stage, resistance-capacity-coupled amplifier and eventually appears across the plates of a cathode ray oscillograph, which has in connection with it a properly synchronized time-axis device. Synchronization is effected by having the hammer close an electrical circuit, which makes the grid of the thyratron go positive, each time the hammer hits the rod,

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the result being that the condenser in parallel with the discharge tube discharges and immediately begins to charge again through the diode in series with it. The potential across the condenser steadily rises, and being impressed on the horizontal deflection plates of the oscillograph, causes the wave-train under examination to be drawn out as a curve with a time base on the oscillo-

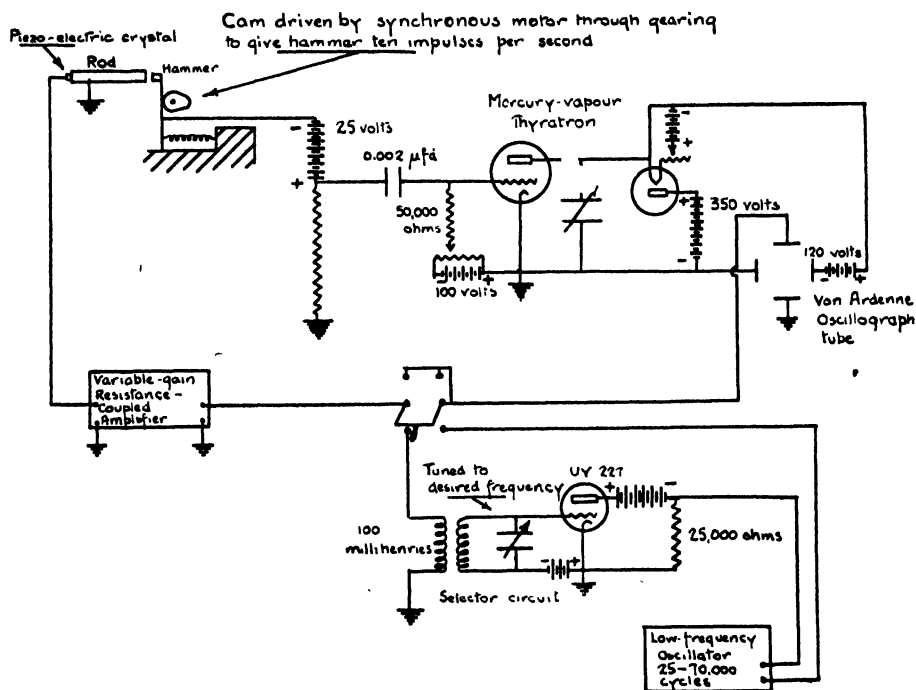


FIG. 1. Schematic diagram of apparatus.

graph screen. Each wave-train is exactly superposed on the one preceding, so that a steady picture is obtained, representative of the periodic displacements of the end of the rod with time.

In general the waves are very complex, being composed of frequencies from several modes of vibration. This complexity has previously been qualitatively observed by other workers (2) at high frequencies. To separate the various components, the voltage from the amplifier is passed through a tuned circuit, and the selected frequency then impressed on the oscillograph. To measure the frequencies thus separated, a heterodyne method is used. The selected frequency is combined with the output from a continuous-wave oscillator, and beats obtained on the oscillograph screen. Originally an aural method of detecting the beats was tried, but the wave-trains are of such short duration that the beat note was obscured by the clicks of the ten wave-trains per second. In the procedure employed, the frequency of the oscillator is varied until zero beat is observed on the screen; this frequency is measured with a wave-meter, and the frequency of the selected component from the complex wave-train is thus also known.

For each wave-train examined, *i.e.*, for every rod, the selector circuit was tuned over the whole band of frequencies from about the lowest fundamental of the rod to a frequency above which the overtones were too weak to be noticed. In this way the fundamentals and overtones of the different modes of vibration for each rod or disk were sorted out and their frequencies measured.

Results

General

In Fig. 2 are shown the various frequencies occurring in rods of duralumin, 6.50 cm. diameter, for lengths from 0.5 to over 40 cm. Curves have been drawn through the plotted points and these curves have been lettered to indicate the mode of vibration which they apparently represent.

F signifies flexural, and L , longitudinal vibrations. The subscript f indicates the fundamental mode and the subscripts 1, 2, 3, etc., the first, second, third and higher overtones. P_1 and P_2 are what have been called in this paper the "plate" frequencies, and C_1 is the "constant" frequency. As far as is known this is the first time these

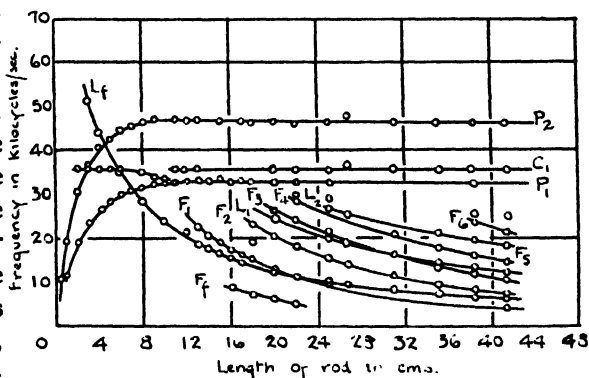


FIG. 2. Curves showing frequencies occurring in duralumin rods, 6.50 cm. in diameter.

last three types of vibration have been observed in rods. These various modes will be discussed in detail later.

By referring to the curves, it will be observed that the flexural and longitudinal frequencies did not normally appear at frequencies greater than about 30 kilocycles. For very short rods (approaching disk characteristics), the fundamental longitudinal vibration was observed up to just over 50 K.C., but for what might really be called "disks" or "plates" only the plate frequencies appeared.

Importance of Point of Impact

Contrary to expectations, within limits, the point where the rod was hit did not appear to make a great deal of difference. It was found that if the rod were struck perpendicularly to the long axis, on the side near one end, the flexural vibrations were very prominent, and the longitudinal ones were weak or absent; but if the impact were anywhere on the end face, the longitudinal and flexural modes appeared with amplitudes nearly independent of the angle at which the hammer struck or the actual point of impact.

Working with disks of large diameter, *i.e.*, 10 cm., it was found that more frequencies appeared if the disk were hit slightly off centre than if the impact occurred exactly at the centre.

With these slight exceptions, the point of impact was not very important in determining whether certain frequencies would or would not appear.

Longitudinal Vibrations

It was hoped originally that the fundamental longitudinal mode would appear for the very short rods, so that wave velocities could be calculated for fairly high frequencies, but as the rods were shortened, the longitudinal vibration disappeared, and hence these calculations could not be made. It has therefore, by this method, not been possible so far to obtain wave velocities for very large values of the ratio, $\frac{\text{radius of rod}}{\text{length of rod}}$ (2), as it had been hoped to do at the beginning of this work.

Flexural Vibrations

The frequencies of the flexural vibrations corresponding to different lengths of rod have been re-plotted in Fig. 3. In the same figure have been drawn lines representing the frequencies of the different flexural modes of a free-free bar, using the theoretical relation between frequency and length of bar as given by Lamb (4, p. 126), i.e., $f = \frac{K\pi}{2} \cdot \frac{k^2}{l^2} \cdot \sqrt{\frac{E}{\rho}}$, where f = frequency,

K = radius of gyration of cross-sectional area, $k = s + \frac{1}{2}$, approximately, where s = an integer, l = length of rod, E = Young's modulus and ρ = density.

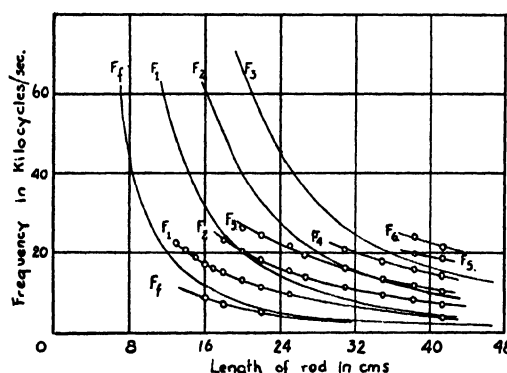


FIG. 3. Flexural vibrations for different lengths of duralumin rods, each 6.50 cm. in diameter.

It will be seen that the theoretical and experimental curves agree for the graver modes for long rods, but as the overtones become higher or the length of rod decreases, the two sets of curves separate widely.

In the derivation of the theoretical frequency equation, certain simplifications were introduced which make the equation applicable only to rods where the wave-length is large compared with the radius. This explains the discrepancy between the theory and experiment.

The equations given by Love (5, p. 288), however, should apply to rods of any radius, and recent experimental work* by Röhrich (10) seems to confirm this for rods when the wave-length of the vibration is small compared with the length. When this last restriction is removed, however, the solutions given by Love for the general equations do not seem adequate, apparently because such solutions are not sufficiently complete. In Part II it will be shown that no solution of the form suggested will satisfy the boundary conditions, one much more complicated apparently being required.

*Although Röhrich did not compare his experimental work with the theory given by Love and amplified by Ruedy (12), if such be done it will be found that the agreement is as good as can be expected.

Plate Vibrations

In Figs. 4, 5, and 6 are shown the plate frequencies plotted for short rods of duralumin of diameters 5, 6.5 and 10 cm. They have been referred to as "plate" vibrations because it is believed that they represent the two normal modes which are usually considered in the theory of the vibrations of a free circular disk. This seems likely because they were the only modes appearing for very thin disks, and the variation of frequency with length for thin disks was linear as predicted by theory. It is suggested that P_1 is the gravest of the normal modes, having two nodal diameters and no nodal circle. P_2 would then be the mode having no nodal diameter and only one nodal circle.

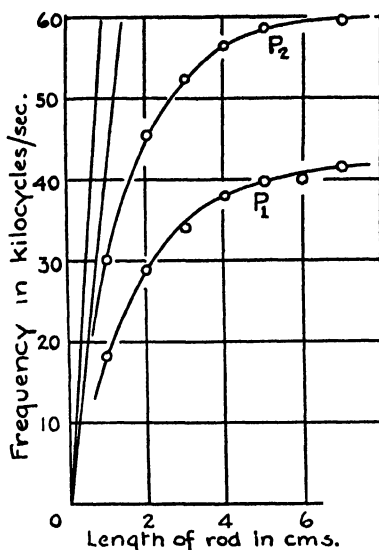


FIG. 4. Plate frequencies for duralumin rods, 5 cm. in diameter.

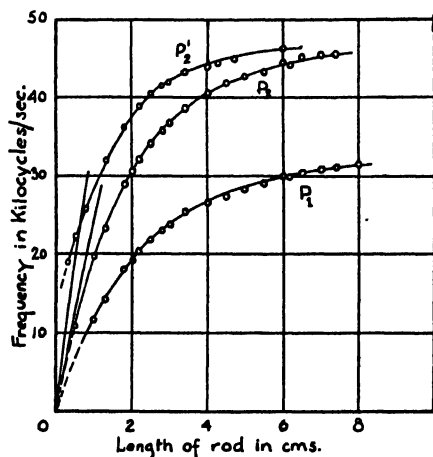


FIG. 5. Plate frequencies for duralumin rods, 6.50 cm. in diameter.

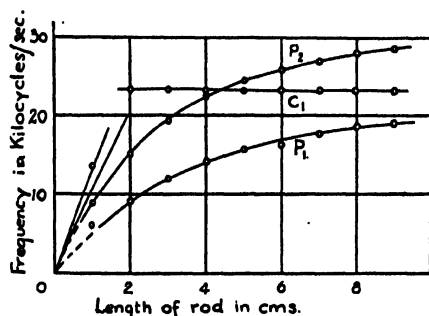


FIG. 6. Plate frequencies for duralumin rods, 10.0 cm. in diameter.

For the disks of duralumin these frequencies were very strong, becoming weaker as the thickness of disk increased. It is, however, remarkable that such vibrations could be detected for rods over 40 cm. long. It is also worth noting that these frequencies are unchanged for different lengths of rod after the "very short rod" stage has been passed.

In Fig. 7 it is shown that by plotting the product of length of rod (l) and frequency (n) against l , a straight line is obtained, except for a slight deviation near the origin.

By means of the theoretical formulas due to Poisson, Kirchhoff, etc., and summarized by Lamb (4, pp. 155-156), the frequencies of the two gravest modes of free vibration in a circular plate have been calculated for various thicknesses. They are represented in Figs. 4, 5 and 6 by the straight lines going through the origin. In deriving these formulas, the assumption is always made that the plates are very thin; hence the results can be used only when this is true. For this reason, the

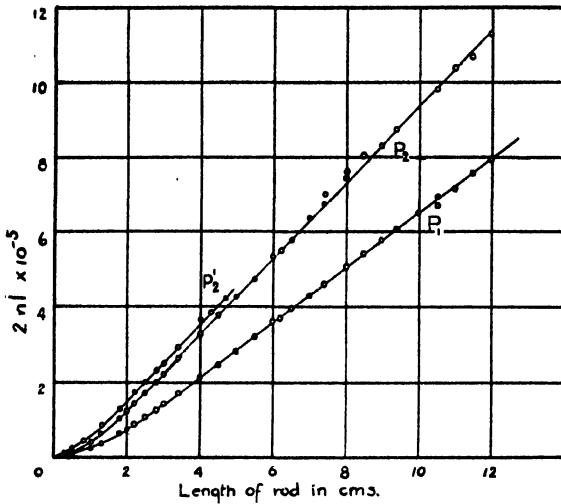


FIG. 7. Showing straight lines obtained by plotting $2nl$ against l .

experimental curves cannot be expected to agree with the theoretical ones over very much of their length. They should, however, become tangent to them for lengths of rods approaching zero. From the graphs, it appears that they do not do so, and the following analysis shows this to be true.

It has been found that the experimental plate frequencies are given by the empirical expressions,

$$f_1 = A_1/a \left(1 - e^{-\frac{k_1 l}{a}} \right),$$

$$f_2 = A_2/a \left(1 - e^{-\frac{k_2 l}{a}} \right),$$

where f_1 and f_2 are the observed plate frequencies; A_1 , A_2 , k_1 , k_2 are constants; l is the length of the rod and a the radius.

When l is small, these equations reduce to,

$$f_1 = A_1 k_1 l/a^2,$$

$$f_2 = A_2 k_2 l/a^2.$$

If the values for the A 's and k 's, as determined for duralumin, be filled in, we obtain,

$$f_1 = 1.50 \times 10^6 \cdot l/a^2 = 0.288 l/a^2 \sqrt{\frac{E}{\rho}}$$

$$f_2 = 2.66 \times 10^6 \cdot l/a^2 = 0.512 l/a^2 \sqrt{\frac{E}{\rho}}.$$

The values for f_1 and f_2 , according to Kirchoff and Poisson, as given by Lamb (4, p. 155-156), are,

$$f_1 = 0.517 l/a^2 \cdot \sqrt{\frac{E}{\rho}}$$

$$f_2 = 0.88 l/a^2 \cdot \sqrt{\frac{E}{\rho}}.$$

It will be seen that the calculated values for f_1 and f_2 are very different from those which have been found experimentally. It may be that the observed frequency, f_2 , corresponds to the theoretical one, f_1 , but the difficulty in this assumption is that the observed frequency, f_1 , is not then accounted for at all by the theory, which indicates that no mode graver than the theoretical f_1 is to be expected.

Underlying the above argument there was the assumption that the equations, $f = A/a \left(1 - e^{-\frac{kl}{a}} \right)$, would apply for plates thinner than those on which

observations were made. It might be argued that the theoretical relations are valid only for very thin plates (thinner than those examined experimentally), and that if observations had been made on such very thin disks, better agreement would have resulted. Upon examining the graphs, however, it will be seen that for the experimental curves ever to become tangent to the theoretical ones a most improbable lateral shift of such curves would be required.

Effect of the Crystal Detector on the Plate Frequencies

Originally a slab of tourmaline, 0.5 cm. thick, covering most of the end of the rod, was employed as a detector, but it was felt that probably the frequencies of the normal modes were being altered considerably by having such a heavy mass cemented to the rod. Accordingly, smaller and smaller pieces of crystal were successively tried, until no change in the frequency of the vibration resulted from further decreasing the size of the detector. The crystal finally used was quartz, 0.257 cm. thick, having an area of about 2.6 cm.², *i.e.*, covering about 8% of the end area of the rods that were 6.5 cm. in diameter. It was found that the frequencies were five to ten kilocycles higher for the tourmaline on the end of the rods than for the thin quartz.

The effect of the detector on frequency is shown in Fig. 5, where the curve P_2 is for the small piece of quartz and the curve P'_2 for the comparatively heavy detector of tourmaline. It will be noticed that the curve P'_2 approaches the curve P_2 for long lengths of rod, and that the straight line obtained by plotting $2nl$ against l for P'_2 in Fig. 7 is parallel to the straight line obtained in a similar way for P_2 . These two facts simply imply that the tourmaline increases the effective length of the rod, which is of course the result one would expect.

Constant Frequency Vibrations

These vibrations, having frequencies independent of length of rod, were observed for duralumin rods and disks of diameters 10.0 and 6.5 cm., but not for the 5.0-cm. rod. In the latter case, the "constant frequency" vibrations were probably at a fairly high frequency, and it was found that in general vibrations did not appear if they were to be expected at frequencies high in comparison with those of the lower modes.

For 10-cm. rods the frequency C_1 had a value of 23.2 K.C., and for the 6.5-cm. rods the frequency was about 35.5 K.C. These frequencies are inversely proportional to the diameter of the rods, so that we can write for them, $f_{c_1} = K/d$, where f_{c_1} is the frequency, d the diameter of the rod and K a constant, presumably depending on the material of which the rod is composed.

Just where these modes of vibration have their origin is not definitely known. It is possible that they are radial vibrations, which affect the crystal at the end of the rod by means of the coupling which exists between the various degrees of freedom. The theoretically calculated frequencies (11) of the fundamental modes of radial vibration for the two cases (10.0 and 6.5 cm.

diameter) are, however, 44.6 and 70.2 K.C., which are almost exactly double the frequencies C_1 . It may be that the theory is in error, and that these frequencies really do arise in a radial vibration, for it is significant that the C_1 vibrations are dependent on the radius and independent of the length. Since the theoretical frequencies bear the 2 : 1 ratio to the experimental ones, it was at first thought that a factor of 2 might have been dropped somewhere in the theory, but examination has failed to reveal any mistakes of this nature.

Other Metals Tried

A few rods of copper and lead were examined, and the observed frequencies compared with those calculated from theory.

With the lead rods so many frequencies appeared that it was not found possible to sort them out and ascribe them to the correct modes of vibration. It was difficult to understand how so many could arise in the known degrees of freedom, unless the velocity of sound in the material were much lower than that usually quoted and the frequencies observed represented a great many of the higher overtones. No satisfactory explanation for this difficulty with lead rods has been found.

PART II

Introduction

Since the simple equation for transverse vibrations in rods given by Lamb (4, p. 126) was found not to agree with the experimental results, an examination was made of the more complete theory given by Love (5, p. 291) to see if it would explain the results obtained in this investigation for comparatively short rods.

General Equations of Vibration

The equations of vibration (5, p. 288) referred to cylindrical co-ordinates are,

$$\begin{aligned} & \alpha^2 \left[\frac{\partial^2 u_r}{\partial r^2} + \frac{1}{r} \frac{\partial u_r}{\partial r} - \frac{1}{r^2} \frac{\partial u_\theta}{\partial \theta} - \frac{u_r}{r^2} \right] + \beta^2 \left[\frac{\partial^2 u_z}{\partial r \cdot \partial z} + \frac{1}{r} \frac{\partial^2 u_\theta}{\partial r \cdot \partial \theta} \right] \\ & + \gamma^2 \left[\frac{\partial^2 u_r}{\partial z^2} + \frac{\partial^2 u_z}{\partial r \cdot \partial z} + \frac{1}{r} \frac{\partial^2 u_\theta}{\partial r \cdot \partial \theta} - \frac{1}{r^2} \frac{\partial u_\theta}{\partial \theta} + \frac{1}{r^2} \frac{\partial^2 u_r}{\partial \theta^2} \right] = \frac{\partial^2 u_r}{\partial t^2} \\ & \alpha^2 \left[\frac{1}{r^2} \frac{\partial^2 u_\theta}{\partial \theta^2} + \frac{1}{r^2} \frac{\partial u_r}{\partial \theta} \right] + \beta^2 \left[\frac{1}{r} \frac{\partial^2 u_z}{\partial \theta \cdot \partial z} + \frac{1}{r} \frac{\partial^2 u_r}{\partial \theta \cdot \partial r} \right] \\ & + \gamma^2 \left[\frac{\partial^2 u_\theta}{\partial z^2} + \frac{\partial^2 u_z}{\partial r^2} + \frac{1}{r} \frac{\partial^2 u_z}{\partial \theta \cdot \partial z} + \frac{1}{r} \frac{\partial u_\theta}{\partial r} - \frac{u_\theta}{r^2} + \frac{1}{r^2} \frac{\partial u_r}{\partial \theta} + \frac{1}{r} \frac{\partial^2 u_r}{\partial \theta \cdot \partial r} \right] = \frac{\partial^2 u_\theta}{\partial t^2} \\ & \alpha^2 \frac{\partial^2 u_z}{\partial z^2} + \beta^2 \left[\frac{\partial^2 u_r}{\partial r \cdot \partial z} + \frac{1}{r} \frac{\partial^2 u_\theta}{\partial \theta \cdot \partial z} + \frac{1}{r} \frac{\partial u_r}{\partial z} \right] \\ & + \gamma^2 \left[\frac{1}{r} \frac{\partial u_r}{\partial z} + \frac{\partial^2 u_r}{\partial r \cdot \partial z} + \frac{\partial^2 u_z}{\partial r^2} + \frac{1}{r} \frac{\partial u_z}{\partial r} + \frac{1}{r} \frac{\partial^2 u_\theta}{\partial \theta \cdot \partial z} + \frac{1}{r^2} \frac{\partial^2 u_z}{\partial \theta^2} \right] = \frac{\partial^2 u_z}{\partial t^2} \end{aligned}$$

$$\text{where } \alpha^2 = \frac{\lambda + 2\mu}{\rho}, \beta^2 = \frac{\lambda}{\rho} \text{ and } \gamma^2 = \frac{\mu}{\rho}.$$

The stress components are,

$$\begin{aligned}\widehat{r\theta} &= \gamma^2 \rho \left[\frac{\partial u_\theta}{\partial r} - \frac{u_\theta}{r} + \frac{1}{r} \frac{\partial u_r}{\partial \theta} \right] & \widehat{zr} &= \gamma^2 \rho \left[\frac{\partial u_r}{\partial z} + \frac{\partial u_z}{\partial r} \right] \\ \widehat{z\theta} &= \gamma^2 \rho \left[\frac{\partial u_\theta}{\partial z} + \frac{1}{r} \frac{\partial u_z}{\partial \theta} \right] & \widehat{zz} &= \alpha^2 \rho \frac{\partial u_z}{\partial z} + \beta^2 \rho \left[\frac{\partial u_r}{\partial r} + \frac{1}{r} \frac{\partial u_\theta}{\partial \theta} + \frac{u_r}{r} \right] \\ \widehat{rr} &= \alpha^2 \rho \frac{\partial u_r}{\partial r} + \beta^2 \rho \left[\frac{\partial u_z}{\partial z} + \frac{1}{r} \frac{\partial u_\theta}{\partial \theta} + \frac{u_r}{r} \right].\end{aligned}$$

As a solution of the general equations, applicable to the case of transverse vibrations, the following is suggested by Love (5, p. 291) for the particle displacements,

$$(u_r, u_\theta, u_z) = (U \cos \theta, V \sin \theta, W \cos \theta) e^{i(\delta z + pt)}$$

where U , V and W are functions only of r .

Now δ may be real or imaginary, so that (u_r, u_θ, u_z) may be either circular or real exponential (which includes hyperbolic) functions of z . It will be shown later that by virtue of the boundary conditions they must be either one type of function or the other; they cannot be combinations of real exponential and circular functions.

Let us consider these two cases, to determine which, if either, is applicable to the present problem.

1. Real Exponential or Hyperbolic Function of z

For this solution the vibrating rod may take up the forms given by the curves e^{-kz} , e^{+kz} , $\sinh kz$ or $\cosh kz$. For a wave travelling down a long rod, nodes are experimentally observable at distances one-half wave-length apart. Obviously the forms for the curve taken up by the rod as given by the above suggested functions could not give such a series of nodes. While such curves for a finite rod are conceivable for the gravest mode of free vibration, no overtones are then theoretically possible.

It may also be shown that such solutions do not satisfy the boundary conditions at $z=0$ and $z=l$. For such values of z we must have $\widehat{zz} = \widehat{z\theta} = \widehat{zr} = 0$.

$$\widehat{zz} = \left[\alpha^2 \rho W \frac{\partial f_1(z)}{\partial z} + \beta^2 \rho \left(\frac{\partial U}{\partial r} \cdot f_2(z) + \frac{V}{r} \cdot f_3(z) + \frac{U}{r} \cdot f_3(z) \right) \right] \cos \theta$$

where $f_1(z), f_2(z), f_3(z) = e^{\pm kz}$, $\cosh kz$ or $\sinh kz$.

Try $f_1 = \cosh kz, f_2 = f_3 = \sinh kz$.

Then, $\widehat{zz} = \rho \left[\alpha^2 kW + \beta^2 \left(\frac{\partial U}{\partial r} + \frac{U+V}{r} \right) \right] \cos \theta \sinh kz \begin{cases} = 0, & \text{for } z = 0 \\ \neq 0, & \text{for } z = l. \end{cases}$

$$\widehat{z\theta} = \gamma^2 \rho \left(kV - \frac{W}{r} \right) \sin \theta \cosh kz \neq 0, \text{ for } z = 0 \text{ or } l.$$

Also, $\widehat{zr} \neq 0$, for $z = 0$ or l .

It may be shown that none of the suggested forms for $f_1(z)$ and $f_2(z)$ can make the stress components vanish at the boundary.

2. Circular Functions of z

The rod may take up the forms given by the curves $\cos kz$ or $\sin kz$. This solution will account for overtones and indicates a series of nodes for a wave travelling down a rod.

Consider the boundary conditions, assuming that $f_1(z) = \cos kz$ and $f_2(z) = \sin kz$.

$$\widehat{zs} = \rho \cos \theta \left[-\alpha^2 kW + \beta^2 \left(\frac{\partial U}{\partial r} + \frac{U+V}{r} \right) \right] \sin kz = 0, \text{ for } z = 0 \text{ and } l, \text{ if } k = \frac{n\pi}{l}.$$

$$\widehat{z\theta} = \gamma^2 \rho \left(kV - \frac{W}{r} \right) \cos kz \sin \theta \neq 0 \text{ for } z = 0 \text{ and } l, \text{ with } k = \frac{n\pi}{l}.$$

It may also be shown that \widehat{zr} will not vanish at the ends of the rod.

For a very long cylinder, *i.e.*, long in comparison with the distance between nodes, the fact that all the stress components do not vanish at the ends may probably be neglected, since it is likely that only a small portion of the rod near each end will be affected. This conclusion has been born out by experiment (10). When this is true, the phase velocity of the wave in the rod is given by the relation, $c = f\lambda = \frac{2lf}{n}$, where f is the frequency of the n th mode.

If this relation be used to calculate velocities from the data obtained for short and medium rods in this research, Fig. 8 is obtained. The theoretical curve based on the above has been drawn in, and the experimental values obtained have been plotted. Each series of points connected by the curves is for a certain mode of vibration, the first, second, third, etc. It will be seen that as the overtones get higher the theoretical velocity curve is more and more closely approached, but for the first few modes the agreement is very poor.

From these results it is clear that solution No. 2 is not applicable to short or medium rods, that is, rods whose lengths are comparable with the distance

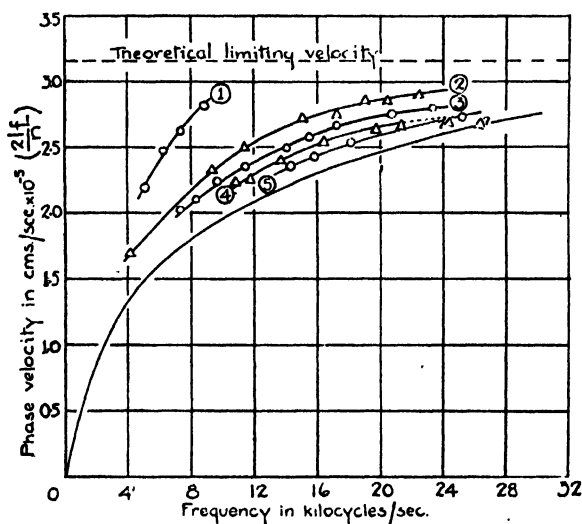


FIG. 8. Theoretical and experimental phase velocities of flexural vibrations in 6.50 cm. diameter duralumin rods.

between successive nodes, though it is sufficiently accurate for long ones. In the theory given by Rayleigh (9) and Lamb (4, p. 126), the particle displacements are found to be combinations of hyperbolic and circular functions of z , *i.e.*, $u_z = A(\cos mz + \cosh mz) + B(\sin mz + \sinh mz)$. The nodes near the ends are then not an equal distance apart, but near the middle of the bar the spacing is uniform. This explains the experimental result depicted in Fig. 8, that when the rod is long and the overtones are high (so that most of the nodes may be considered remote from the

ends), very little error is introduced by considering all the nodes equally spaced, that is, assuming that the displacement of the particles in the rod is given by a sine function. When the number of nodes is small, however, most of the nodes are not equally spaced, the displacement of points on the rod cannot be represented by a sine function, and hence the calculations of phase velocity depending on this hypothesis are not valid.

It will now be shown that the general equations of vibration cannot have a solution applicable to a cylindrical rod of the form,

$$(u_r, u_\theta, u_z) = (U \cos \theta, V \sin \theta, W \cos \theta) f(z) e^{i\omega t},$$

where $f(z)$ is a combination of both hyperbolic and circular functions. It will also be shown that if each of (u_r, u_θ, u_z) can be expressed as $F_1(\theta) \cdot F_2(r) \cdot F_3(z) \cdot e^{i\omega t}$, that is, products of functions, each of which is a function only of r, θ or z , then $F_3(z)$ must be a real or imaginary exponential function.

Let us take,

$$(u_r, u_\theta, u_z) = U \cos \theta \cdot F_1(z), V \sin \theta \cdot F_2(z), W \cos \theta \cdot F_3(z) \cdot e^{i\omega t}.$$

Only one of the general equations need be considered, *i.e.*,

$$\begin{aligned} \alpha^2 \left[\frac{\partial^2 U}{\partial r^2} + \frac{1}{r} \frac{\partial U}{\partial r} - \frac{U}{r^2} \right] F_1 - \alpha^2 \cdot \frac{V}{r^2} \cdot F_2 + \beta^2 \left[\frac{\partial W}{\partial r} \cdot \frac{\partial F_2}{\partial z} + \frac{1}{r} \frac{\partial V}{\partial r} \cdot F_2 \right] \\ + \gamma^2 \left[U \frac{\partial^2 F_1}{\partial z^2} + \frac{\partial W}{\partial r} \cdot \frac{\partial F_2}{\partial z} + \left(\frac{1}{r} \cdot \frac{\partial V}{\partial r} - \frac{V}{r^2} \right) F_2 - \frac{1}{r^2} \cdot U F_1 \right] = -\rho^2 U F_1 \end{aligned} \quad (1)$$

At $r=a$, the conditions $\widehat{zr}=0$, and $\widehat{rr}=0$ must be satisfied;

$$\text{that is, } \left(U(a) \cdot \frac{\partial F_1}{\partial z} + \frac{\partial W}{\partial a} \cdot F_2 \right) \cos \theta = 0 \quad (2)$$

$$\text{and } \left[\alpha^2 \frac{\partial U}{\partial a} \cdot F_1 + \beta^2 \left(W(a) \frac{\partial F_2}{\partial z} + \frac{F_1}{a} \right) \right] \cos \theta = 0 \quad (3)$$

Of course, the general equation (1) must hold also for $r=a$.

From (2), since $U(a)$ and $\frac{\partial W}{\partial a}$ are not in general each zero, we must have

$$\frac{\partial F_1}{\partial z} = k_1 F_2, \text{ so that then } \left(k_1 U(a) + \frac{\partial W}{\partial a} \right) = 0.$$

From Equation (3), likewise,

$$\frac{\partial F_2}{\partial z} = k_2 F_1.$$

Substituting for $\frac{\partial F_2}{\partial z}$ in (1), we get,

$$\begin{aligned} \alpha^2 \left[\frac{\partial^2 U}{\partial r^2} + \frac{1}{r} \frac{\partial U}{\partial r} + \left(\frac{\beta^2}{\alpha^2} - \frac{\alpha^2 + \gamma^2}{\alpha^2} \cdot \frac{1}{r^2} \right) U + (\beta^2 + \gamma^2) k_2 \frac{\partial W}{\partial r} \right] F_1 \\ + \left[(\beta^2 + \gamma^2) \frac{1}{r} \frac{\partial V}{\partial r} - (\alpha^2 + \gamma^2) \frac{V}{r^2} \right] F_2 + \gamma^2 U \frac{\partial^2 F_1}{\partial z^2} = 0 \end{aligned} \quad (5)$$

The coefficient of $\frac{\partial^2 F_1}{\partial z^2}$ cannot be zero, since for $U=0$, there would be no movement radially of the particles in the rod, and for a transverse vibration such must occur.

The coefficient of F_2 might be zero, in which case we should have $V = Cr^n$, where C is a constant of integration and $n = \frac{\alpha^2 + \gamma^2}{\beta^2 + \gamma^2}$. If, however, one of the other equations, e.g., Equation 3, be considered, it will be found that some other function of V would be required to make the coefficient of F_2 vanish. Hence, in Equation 5, the coefficients of neither F_2 nor $\frac{\partial^2 F_1}{\partial z^2}$ can be made to vanish individually, so that we must have,

$$F_2 = k_4 F_1 \quad \text{and,} \quad \frac{\partial^2 F_1}{\partial z^2} = k_4 F_1.$$

The solution of this last equation is $F_1 = Ae^{k_4 z} + Be^{-k_4 z}$, where k_4 may be either real or imaginary, since k_4 is an undetermined constant. It will be seen that a solution cannot exist of the form $Ae^{\pm k_4 z} + Be^{\pm k_4 z}$, which means that F_1 cannot be a combination of both circular and hyperbolic functions.

The above results show that if (u_r, u_θ, u_z) can be expressed as products of functions, each of which is a function only of r, θ or z , then $F_1(z)$ must be an exponential function with either real or imaginary argument. It has previously been shown that neither of these two cases leads to a solution of the general equations which is applicable to the case of transverse vibrations in short rods, and therefore it has been proved that (u_r, u_θ, u_z) cannot be expressed as products of separable functions of the three variables. It appears, therefore, that a more complex solution is required.

It is possible that functions of the form, $r^a(r-a)^m z^l(z-l)^n F_1(r, z) F_2(\theta)$ might be determined which would fit the various equations, but it appears that to find suitable solutions would be very difficult. If general solutions could be determined, it is probable that all the modes of vibration determined experimentally in this investigation, for some of which it has been difficult to account theoretically, might be explained.

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AN ELECTROSTATIC VOLTMETER FOR HIGH VOLTAGES¹

BY GEO. S. FIELD²

Abstract

An electrostatic voltmeter which is of fairly simple construction is described. It has a scale which is more nearly linear than is usually found with such instruments, and which can be applied to several different voltage ranges.

The essential parts of the instrument are shown schematically in Fig. 1. S_1 is a fixed duralumin ball, 15.2 cm. in diameter; S_2 is a light celluloid ball, 3.8 cm. in diameter, covered with aluminium paint. To S_2 is attached a small shaft (shown at a),

which is pivoted in jewel bearings. W_1 and W_2 are weights, the first being used to adjust the sensitivity of the moving element, and the second to correct the zero position of the pointer, P , which is of thin glass and is easily removable.

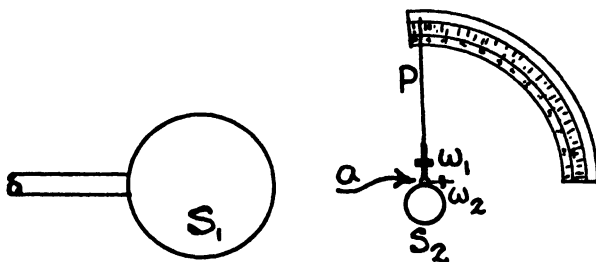


FIG. 1. Diagram showing arrangement of instrument parts.

The ball S_1 is attached to a duralumin rod, fitted into a sleeve, so that it may be moved towards and away from S_2 . S_1 is connected to the high potential. The support which holds the jewel bearings, and hence the moving element, is connected to ground. In order that S_2 may also be grounded, a very fine, loosely coiled piece of gold wire connects it to the support. The housing consists of a mahogany box, fitted with a window through which the moving element may be watched. A photograph of the complete instrument is shown in Fig. 2.

If E be the potential difference between the two balls, the force tending to draw them together is kE^2/d , where k is a constant and d is the distance between their centres. In this expression the distortion of the electric field as the result of other material, *e.g.*, the metal frame holding up the moving element, being in the neighborhood of S_1 and S_2 is neglected. If l be the distance from the centre of S_2 to the axis of the moving element, and if θ be the angle through which S_2 has turned about this axis, the torque tending to rotate S_2 is $(kE^2/d^2) l \cos \theta$.

If m be the mass of the moving element, and t the distance of the



FIG. 2. Photograph of completed instrument.

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Contribution from the National Research Laboratories, Ottawa, Canada.

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centre of gravity from the axis, the balancing torque due to the weight of the element is $mgl \sin \theta$, where g is the acceleration due to gravity.

When the element comes to rest, therefore,

$$\text{or,} \quad \begin{aligned} (hE^2/d^2) l \cos \theta &= mgl \sin \theta, \\ E^2 &= K d^2 \tan \theta, \end{aligned} \quad (1)$$

which is the equation of the meter.

In Fig. 3 curves have been drawn through points plotted from experimental results. Curve 1 is for the two balls, 23.5 cm. apart, and Curve 2 is for the balls 49.5 cm. apart. From one point on Curve 1, the corresponding relation

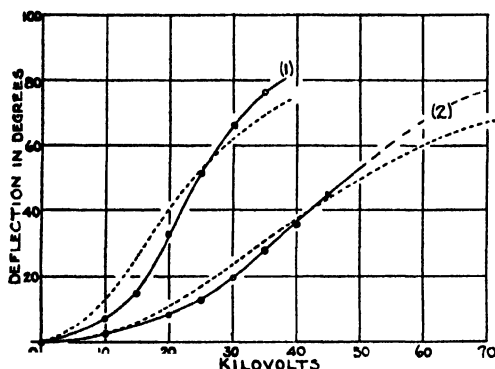


FIG. 3. Theoretical and experimental curves.

between E and θ was used to determine K in Equation (1). The broken curve was then drawn by using the theoretical relation. Using the same equation, with the appropriate d supplied, the second broken curve was drawn. That the electric field is considerably distorted is shown by the discrepancy between the theoretical and experimental curves. In the second case the agreement is better, which suggests that the field becomes more regular as the spheres are separated.

In Fig. 4 is shown a facsimile of the scale of the meter for the two calibration curves mentioned above. As voltages above 45,000 were not available,

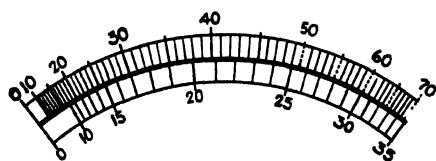


FIG. 4. Instrument scales.

it was not possible actually to calibrate above that voltage. However, probable scale divisions have been drawn in, based on an extrapolation of Curve 2. It will be seen that the scale is much more linear than is usually obtained with electrostatic voltmeters,

and that if the theoretical curves could be more closely approached, even greater linearity would be obtained.

THE SOLUBILITY OF LIME IN WATER AND THE SPECIFIC CONDUCTIVITIES OF ITS SATURATED SOLUTIONS¹

BY A. D. GRIEVE,² G. W. GURD,³ and O. MAASS⁴

Abstract

The specific conductivities of saturated solutions of calcium hydroxide have been measured from 0° to 130° C. Special precautions were taken to eliminate errors due to impurities. The degree of dissociation and so called dissociation constants have been calculated for these solutions from 0° to 100° C. These data have been used to calculate a new set of solubility data for calcium hydroxide at temperatures from 0° to 25° C. Solubilities of calcium hydroxide have been measured over this temperature interval by an indirect method, and the results are in fair agreement with the calculated values.

Introduction

As a preliminary to an investigation of the equilibria existing in sulphite cooking liquor (the system $\text{CaO-H}_2\text{O}$), the specific conductivity of saturated solutions of calcium hydroxide over the temperature range 0° to 130° C. was required. A survey of the literature revealed that the only previous measurements on conductivities of calcium hydroxide were a series of measurements by Ostwald (9) on unsaturated solutions at 25° C. and measurements by Miller and Witt (7) on saturated solutions at 30° C. The former are admitted by the author to be subject to the experimental error introduced by carbon dioxide contamination. It was decided to measure the specific conductivity of saturated solutions of calcium hydroxide over the entire range from 0° to 130° C., employing a technique ensuring freedom from errors due to contamination by impurities. The data obtained have led to the calculation of a new set of values for the solubility of calcium hydroxide between 0° and 25° C., at which temperatures the previous determinations of the solubility are in very poor agreement with one another.

Apparatus

The cell in which the conductivity measurements were made is shown diagrammatically in Fig. 1. It was made of Pyrex and contained a stirrer *S*, also made of Pyrex, which was actuated by two electromagnets *M*₁ and *M*₂, permitting stirring within the completely enclosed cell. The stirrer was

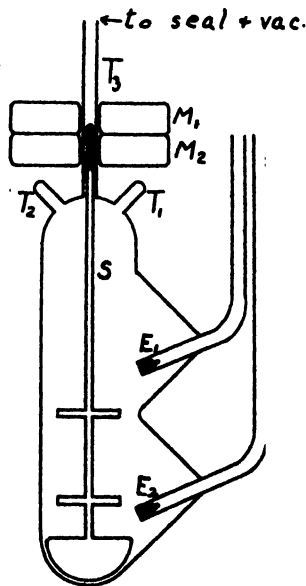


FIG. 1. Conductivity cell.

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Contribution from the Physical Chemistry Laboratory, McGill University, Montreal Canada.

² Demonstrator, Department of Chemistry, McGill University, at the time of investigation.

³ Holder, at the time, of a studentship under the National Research Council of Canada.

⁴ Macdonald Professor of Physical Chemistry, McGill University.

designed to give good agitation, and keep the excess solid in complete suspension. The electrodes E_1 and E_2 were of platinum foil, sealed in the end of Pyrex tubes, which in turn were sealed into "bay windows" in the side of the cell. The electrode assembly as well as the other features of the cell were all designed with a view to preventing solid from depositing in places where it would not be subject to the action of the stirrer. The side arm, T_1 , when opened, was used to introduce calcium oxide. Water was distilled into the cell through T_2 . T_3 served as a guide for the stirrer and also led to the vacuum pump; it could be closed by means of a mercury seal.

The cell was immersed in a constant-temperature bath in which water was used at low temperatures and oil at high temperatures. To prevent condensation of water in the exposed portion of the tube T_3 , it was wound with resistance wire, which could be electrically heated, and covered with asbestos, in order that the temperature of the tube could be kept above that of the bath.

The electrodes were platinized and the cell constant determined in the usual way. There was a small lead resistance due to the thinness of the platinum foil of the electrodes and the leads to the bridge. This was measured by short-circuiting the electrodes with mercury and measuring the cell resistance under these conditions.

In making all resistance measurements a Leeds and Northrup helical slide wire was used. It, as well as the resistance box, had been previously checked and found satisfactory. A Vreeland oscillator, operating at a frequency of 1000 cycles, supplied the current. The null point was detected by means of ear phones.

Materials

Calcium oxide was prepared from Iceland spar by heating the ground crystals in a furnace at 100° C. for several days. It was tested for freedom from carbon dioxide in two ways: first, by dissolving a sample in acid and bubbling any gas produced into barium hydroxide; second, by covering a small lump with gelatin and allowing acid to diffuse through the gelatin and react with the lime—any carbon dioxide liberated was trapped beneath the gelatin and could be seen as a bubble, with aid of a microscope. No carbon dioxide was found by either of these methods. Distilled water was freed of dissolved gases by a series of cycles of freezing (in carbon dioxide snow), evacuating, and melting. It was then slowly distilled into the cell at its own vapor pressure, through the side arm T_2 , which was then sealed off.

Procedure

Lime was placed in the cell in considerable excess of the amount needed for saturation. Water was distilled into the cell, and the solution was stirred until equilibrium was reached, as shown by constancy of the specific conductivity during at least 30 min.; but since equilibrium was reached very slowly it was very difficult to use this method at the higher temperatures. At the higher temperatures, the following procedure was adopted: the temperature

of the bath was raised to the desired value and held there during one hour, conductivity readings being taken every 10 or 15 min. The value obtained at the end of an hour was recorded. The temperature was then raised to the next higher value and the procedure repeated. This gave a series of values obtained with ascending temperatures; a similar series was obtained by decreasing the temperature, from the maximum, step by step. The values obtained by these two methods were averaged to obtain an equilibrium value for the conductivity.

The conductivity values are given in Column 2 of Table I. Column 3 contains a set of values for the solubility of calcium hydroxide. These values were obtained from a curve representative of the average of the data of Maben (6), Lamy (4), Herzfeld (3), Guthrie (1), Moody (8), Haslam, Calingaert and Taylor (2) and Miller and Witt (7). Column 4 contains values for the equivalent conductivity at these concentrations. Column 5 contains values for the equivalent conductivity at infinite dilution, calculated from the data of Kohlrausch (5) for the mobility of the ions. Column 6 gives values for the degree of ionization calculated from the equivalent conductivity data. Column 7 gives values for the dissociation constant.

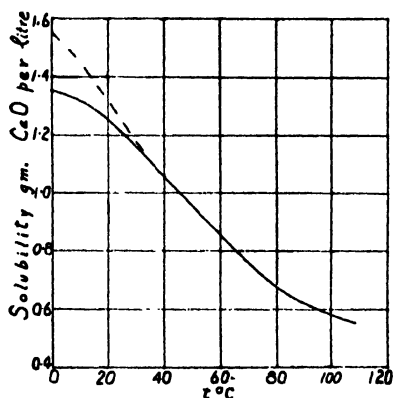


FIG. 3. Relation between solubility and temperature.

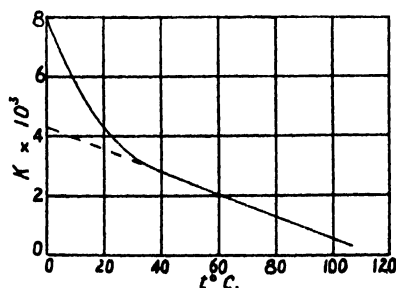


FIG. 4. Relation between dissociation constant and temperature.

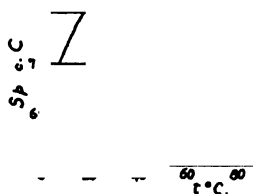


FIG. 2. Relation between specific conductivity of saturated solutions of calcium hydroxide and temperature.

Owing to the scarcity of solubility data above 100° C., none of the values for solubility, λ_c , λ_o , α , nor K are given above that temperature.

The values for the specific conductivity of saturated solutions of calcium hydroxide are plotted against temperature in Fig. 2. The average curve for the solubility of calcium hydroxide as obtained from the literature is represented by the continuous line in Fig. 3; the discontinuous portion of this curve will be discussed later. The values of the dissociation constant are plotted against temperature in Fig. 4. The extrapolation of a portion of this curve will be referred to later.

TABLE I
CONDUCTIVITY VALUES AND RELATED CONSTANTS

t°C.	$k \times 10^3$	Solubility of CaO, gm./litre	λ_0	λ_0	α Degree of ionization	$K \times 10^3$
0	5.85	1.351	121.5	145.9	0.832	7.97
10	7.20	1.322	152.7	189.9	0.804	5.89
18	7.99	1.272	176.1	225.0	0.783	4.55
25	8.50	1.207	197.3	255.5	0.772	3.74
37	9.15	1.086	236.3	308.2	0.767	2.91
50	9.66	0.958	283.0	365.2	0.775	2.43
60	9.82	0.860	319.6	409.0	0.781	2.06
70	9.72	0.764	356.3	453.0	0.787	1.70
80	9.48	0.680	390.0	497.0	0.785	1.33
90	9.14	0.620	413.0	540.7	0.764	0.927
100	8.69	0.578	422.0	584.5	0.722	0.576
110	8.20					
120	7.67					
130	7.12					

Discussion

It will be seen in Fig. 4 that the values of the dissociation constant, when plotted against temperature, give a straight line above 37° C.; below 37° C. however the values increase much more rapidly than would be given by a straight line. It was felt that this rather unexpected break in the curve pointed to some inaccuracy in the data used to calculate K , *viz.*, specific conductivities and solubilities.

The specific conductivities appear to be consistent among themselves, and, further, at 30° C., the only point at which other conductivity data are available, the agreement with the value of Miller and Witt (7) is good.

The solubility data used are not so satisfactory. From about 37° to 100° C. the agreement amongst the various determinations is reasonably good, and the values which have been used probably are quite close to the true value. Below 37° C. agreement is not nearly so good, consequently all values in this temperature range must be regarded with some doubt.

Assuming that the values for solubility and, therefore, for dissociation constant, above 37° C. are correct, the dissociation constant-temperature curve from 37° to 100° C. has been extrapolated to 0° (Fig. 4). From these extrapolated values for K , the values have been calculated for the solubility which would be necessary to give such values for the dissociation constant.

These are given in Table II, and are plotted as a discontinuous curve in Fig. 3.

As a check on these calculated solubilities, the solubility has been determined by the following method:

Specific conductivities of unsaturated solutions of calcium hydroxide, of accurately determined concentration, were measured over this temperature interval, the procedure being the same as that used for saturated solutions. The values obtained at a given temperature for a series of solutions of different

TABLE II
CALCULATED SOLUBILITY VALUES

$t^{\circ}\text{C.}$	$K \times 10^3$ (extrapolated)	$k \times 10^3$	λ_0	Calcd. solubility of CaO, gm./litre
0	4.29	5.85	145.9	1.55
10	3.92	7.20	189.9	1.45
18	3.62	7.99	225.0	1.34
25	3.36	8.56	255.8	1.25

concentration were plotted against concentration. A curve was drawn through these points and extrapolated until it intersected a straight line drawn parallel to the concentration axis and through the conductivity of a saturated solution for that temperature. The concentration at the point of intersection gives the solubility at that temperature.

The conductivity values obtained for these unsaturated solutions are given in Table III.

TABLE III
CONDUCTIVITIES OF UNSATURATED
SOLUTIONS

Con'c.: 0.536 gm./litre		Con'c.: 0.737 gm./litre	
$t^{\circ}\text{C.}$	$k \times 10^3$	$t^{\circ}\text{C.}$	$k \times 10^3$
2.9	2.60	0.1	3.20
11.9	3.18	10.0	3.99
19.7	3.70	19.7	4.87
30.0	4.31	29.7	5.73

TABLE IV
SOLUBILITY OF CALCIUM OXIDE (gm. per litre)

$t^{\circ}\text{C.}$	Extra- polation value	Calcd. value	Av. value from literature
0	1.45	1.55	1.35
10	1.39	1.45	1.32
18	1.30	1.34	1.27
25	1.22	1.25	1.21

The two sets of data in Table III, with the origin, give a series of isothermal curves which are slightly concave toward the concentration axis. The solubility values obtained by extrapolating these curves are given in Column 2 of Table IV. In Column 3 the values calculated previously (Table II) are given. The last column gives the average values in the literature.

A comparison of the values in Table IV shows that the calculated values are more nearly correct than the average of the data from the literature, because it seems likely that the values obtained by the straight line extrapolation mark the lower limit of solubilities and that the true values lie between those given by an extrapolation of a curve and by an extrapolation of a straight line, in which case the calculated values must be very nearly correct.

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A POWER LAUNDRY COMPARISON OF THE DETERGENT EFFICIENCIES OF ALKALINE SOAP BUILDERS¹

BY O. M. MORGAN²

Abstract

Five commonly used alkaline soap builders, namely, caustic soda, soda ash, modified soda, sodium metasilicate, and trisodium phosphate have been tested for detergent efficiency in full-scale power laundry tests. The above sequence represents their decreasing order of efficiency.

Optimum detergent conditions do not occur at the same pH value for each builder nor is the same quantity of soap required to produce effective washing.

All builders studied are free rinsing and produce no loading of the fabrics. Optimum quantities of builder to produce the maximum detergent effect are tabulated.

The problem of comparing different alkalies as soap builders in a full-scale power laundry is one which is beset with many variables and difficulties. Possibly this is the reason why previous experimenters have not put their results on record. One of the main difficulties encountered is the variation in type and intensity of soil on the fabrics to be washed. This soil may be acid or alkaline, usually the former, and the extent of acidity or alkalinity cannot be determined prior to treatment. Its effect on the first suds bath is very marked. In addition to this there occur in the soiled work stains which may or may not be transferable to other fabrics. This is objectionable but unavoidable. Then too there is the personal factor involving the washman. A reliable washman who will carry out his instructions to the letter is of major importance in performing work of this nature.

Through the very kind and whole hearted co-operation of Vail's Laundry, Ottawa, Canada, and Mr. H. G. Vail, facilities were placed at our disposal for the carrying out of the work contained herein. It is not claimed that the results obtained are the last word in this connection. It is felt however that a decided step forward has been made in the study of the actual laundry performance of the common soap builders. No trade preparations have been included in this study. The work has been confined to pure chemicals to which no trade names have been affixed.

Previous Work

In two previous publications (1, 2) the writer has described a method for evaluating and comparing the detergent efficiencies of neutral and built soaps. This work was of a preliminary nature to develop a trustworthy method of making these comparisons and also to determine whether different detergent effects existed. Preliminary figures were obtained as to how pronounced these

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differences were. This work was all carried out on laboratory scale. The results indicated that although full-scale experiments in a power laundry would be long and tedious much information could be gained. It was realized however that by carrying out these experiments in a single laundry the results would bear a certain amount of specificity due to local conditions. By this is meant that from one plant to another conditions of soil, water, climate, and general technique would vary. This difficulty unfortunately cannot be conveniently overcome. Since the work is of a comparative nature many of these factors will cancel out giving more weight to the general nature of the results.

Experimental

Test Bundle

The test bundle used in these experiments consisted of a piece of cotton towelling 17 by 18 in. to which was attached a piece of standardly soiled cloth 9 by 9 in. The towelling was used to determine tensile strength losses. It had a thread count of 60-42 and a tensile strength in the filling of 81 lb. per inch. The soiled strip, as in previous work, was used for photometric measurements.

Wash Wheels

Two wash wheels were used. Each of these was constructed with Monel metal cylinders and shells, and wooden ends. The size of the cylinder was 42 in. in diameter and 84 in. in length with four longitudinal ribs 7 in. in height.

These machines were calibrated for volume of water as follows: an average load of soiled clothes and nets, 245 lb., was placed in the cylinder. Successive additions of water were made with an 8-gal. can. At intervals the wheel was allowed to run until an equilibrium water level was reached. It is these equilibrium levels with the wheel in motion that are recorded in Table I.

TABLE I
VOLUME CALIBRATION OF THE MONEL METAL WASH WHEEL (42 BY 84 IN.)

Water level, in.	4	5	6	7	8	10	12	14
Volume, gal.	110	120	129	138	147	164	182	198

It was very important to know the actual volume of water present at any level in order to be able to calculate the amount of soap and builder to use.

Washing Formula

The washing formula used in this work was the recognized "multiple suds" formula. This consists of four suds baths followed by six rinses. The details of this formula are given in Table II.

TABLE II
"MULTIPLE SUDS" WASHING FORMULA

No.	Operation	Water level, in.	Time, min.	Temp., ° F.	No.	Operation	Water level, in.	Time, min.	Temp., ° F.
1	Break	5	10	100	6	Rinse	8	5	180
2	Suds	4	15	120	7	Rinse	8	5	160
3	Suds	4	15	160	8	Rinse	8	5	130
4	Suds (bleach)	4	15	170	9	Rinse	10	5	100
5	Rinse	4	5	180	10	Blue	12	5	90

Method of Adding Soap

A high quality commercial soap was made up into a 5% liquid soap stock. No builder was added. The analysis of this soap is as follows:—moisture, 2.01; total soap, 97.05; sodium chloride, 0.004; free alkali (as Na_2O), 0.045; glycerol, 0.89%; titer, 39.7°C . The liquid soap was added to the wash wheel in measured quantities.

Method of Adding Alkali and Bleach

The amount of alkali necessary was calculated as a percentage of the weight of water in the wheel. Hence if the amount of water present were 120 gal. and the desired alkali concentration 0.05% the amount of alkali

added would be $1200 \times \frac{0.05}{100} = 0.6 \text{ lb.}$

The alkalies were weighed out into bottles and added to the wash wheel in the dry form prior to adding the soap. Sufficient water was added to the wash wheel to bring it to the desired level before the alkali addition was made. In the case of caustic soda the material was dissolved in a small amount of water and added to the wash wheel very slowly. The same amount of alkali was added to each suds bath. A slightly greater amount was used in the "break" operation due to the higher water level, all concentrations being in weight percentage of the water present. It is realized that the builder additions to the last two suds baths are larger than those commonly found in plant practice. The reason for maintaining the same concentration throughout the sudsing operations was to facilitate obtaining comparative results.

Two quarts of sodium hypochlorite bleach carrying 1.0% available chlorine per 100 lb. of clothes was added in the fourth operation.

Washroom Control

The off wash liquors from each operation in five consecutive washes were titrated against 0.1 *N* nitric acid using both methyl orange and phenolphthalein as indicators. pH determinations were also made using a Taylor long range slide comparator. This instrument was previously checked against electrometric pH measurements on buffer solutions covering the laundry pH range. In this way a careful check was kept on the uniformity of the washing. Data were also obtained on the free rinsing qualities of the various builders and were further substantiated by subsequent ash determinations on the test pieces. This will be discussed in a later section.

Photometric Measurements

After 2, 5, 10, and 20 washes photometric measurements were conducted on the standardly soiled strips using a Zeiss Pulfrich photometer. The increase in brightness of the fabrics is considered as the criterion of washing efficiency.

Tensile Strength Measurements

After the completion of 20 washes tensile strength measurements were made using an Alfred Suter tensile strength machine having breaking ranges of 0-150 and 0-300 lb. Tensile strength losses are given in a later section.

Results

In laboratory experiments previously recorded (2) optimum concentrations of soap builders were determined at which maximum washing efficiency was obtained. In the present experiments concentrations of builder both above and below these optimum concentrations were tried out. The concentrations of builder used are given in Table III in weight percentage.

TABLE III
CONCENTRATION OF BUILDER IN WEIGHT %

Conc. No.	Soda ash	Trisodium phosphate	Modified soda	Sodium metasilicate	Caustic soda
1	0.013	0.03	0.01	0.026	0.004
2	0.026	0.06	0.05	0.052	0.008
3	0.053	0.12	0.10	0.104	0.020
4	0.106	0.24	0.20	0.208	0.040

In each case it was at concentration No. 2 that maximum efficiency was obtained in the laboratory experiments. In Fig. 1, where increasing brightness is plotted against number of washes, it will be noted that concentration No. 2 is again predominant with respect to efficiency in the plant experiments. This may not be the exact concentration for maximum efficiency

but approximates it quite closely. This contention is maintained³ for two reasons. In the first place laboratory and plant concentrations check for the optimum value. In the second place a manufacturer of sodium metasilicate advises the use of 0.33 lb. of this product per 100 lb. of clothes being washed. This value has been obtained by practical experience in the plant and by laboratory test. The amount of metasilicate recommended by the results obtained in this work is 0.27 lb. per 100 lb. of clothes. These values may be considered to be in good agreement when all variables are considered.

From Fig. 1 it is apparent that in the cases of caustic soda, sodium metasilicate, and modified soda, concentration No. 2 is definitely more efficient than the other concentrations used. In the case of trisodium phosphate (indicated as T.S.P.) concentration No. 2 leads the field after 10 and 20 washes which is quite definite proof of its superiority. In the case of soda ash the differences in efficiency are not so pronounced from one concentration to another. Repeated experiments gave similar results. However at the end of 2, 5 and 10 washes concentration No. 2 shows a slight superiority. This slight difference in efficiency at various concentrations for soda ash also showed up in previous work (2) where it was noted that an efficiency peak existed which was not sharp or well defined. In contrast to this, caustic soda exhibited a very sharp and well-defined efficiency peak. In the present work (see Fig. 1) caustic soda shows decided optimum conditions to exist at concentration No. 2, which was the peak concentration in the previous work.

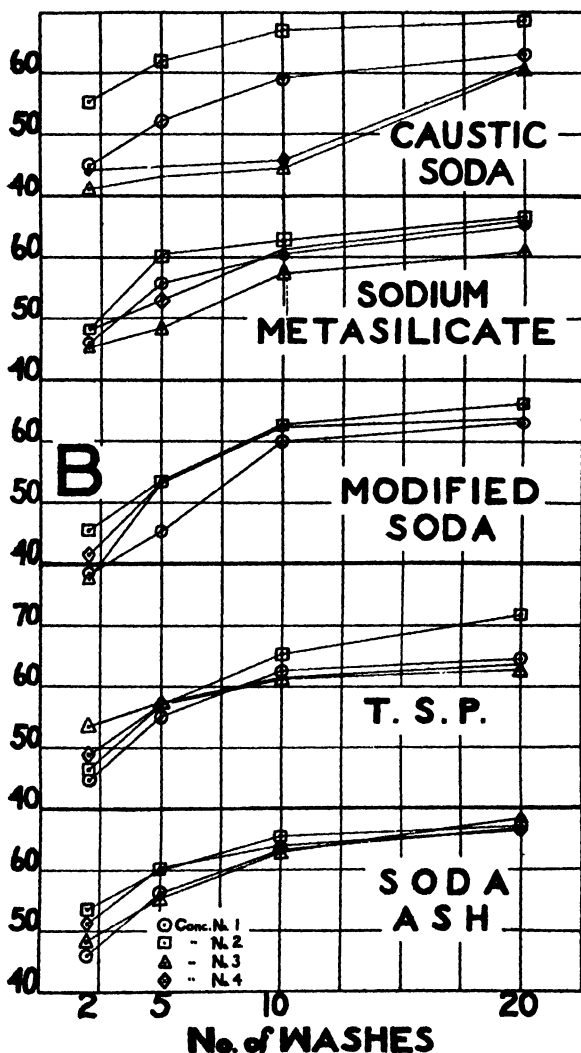


FIG. 1. Increasing brightness of soiled cloth plotted against number of washes.

In Fig. 2 increasing brightness is again plotted against number of washes and the curves of concentration No. 2 for each of the five alkalis are compared for relative efficiency. It will be noted that smooth and nearly parallel

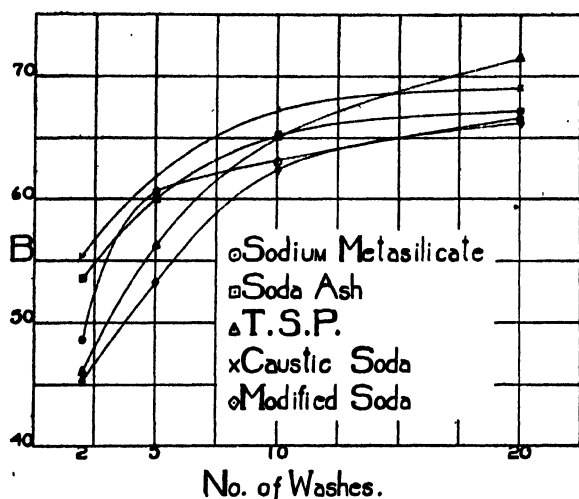


FIG. 2. Relative builder efficiencies compared under optimum conditions.

bundle results?" From the experience gained in this work it has been found that after 2 or 5 washes the results are based to too great an extent on the idiosyncrasies of the individual washes. After 5 washes individual factors begin to disappear and more definite conclusions may be drawn from the 10- and 20-wash data.

After visiting approximately 100 laundries both in Canada and the United States the writer has been impressed by the fact that equally good grades of finished laundered fabrics are being turned out with various combinations of supplies, provided that certain important features of technique are observed. These may be summarized as follows:

- (a) The maintenance of a high suds on the "break" operation.
- (b) The maintenance of a good running suds on the succeeding sudsing operations. Poor quality work is often the result of allowing a suds to fall before the operation is complete.
- (c) The maintenance and proper use of a stock soap solution that is properly blended with respect to soap and alkali.
- (d) Careful temperature control and adequate rinsing facilitated by an ample hot water supply is very important. Water of zero hardness should be used.

In Fig. 2 where optimum conditions of the five alkalis are compared it will be noted that after 10 and 20 washes the difference in efficiencies is not very pronounced, nor did the quality of the actual washing in the plant itself vary to any marked extent. Hence comparisons of the efficiencies of these builders cannot be logically made from a quality standpoint. The proper

curves exist for caustic soda, soda ash, and modified soda, falling in the foregoing order of decreasing efficiency. The curves for trisodium phosphate and sodium metasilicate intersect at certain points. This makes exact comparisons uncertain on some aspects of the case. The broad conclusions regarding these builders are however unaffected.

At this point the following question may be raised: "After how many washes is it practical to make efficiency comparisons when the comparisons are based on test

concentrations of builder to be used must be stressed. A comparison of these concentrations combined with the amount of soap necessary to produce effective washing gives the clearest picture of the situation.

Considering for the moment only actual washing efficiency with a given fixed concentration of soap and optimum conditions as regards concentration of builder, an equal efficiency figure will be ascribed to each builder. Let it be the average brightness figure for 10 washes, which is approximately 65. In Table IV comparative figures for the five builders are presented.

TABLE IV
EFFICIENCY DATA FOR ALKALINE SOAP BUILDERS

Alkali	Efficiency	Alkali conc., %	Alkali efficiency index	Pound efficiency index
Caustic soda	65	0.008	8125	1
Soda ash	65	0.026	2500	3.2
Sodium metasilicate	65	0.052	1250	6.5
Trisodium phosphate	65	0.06	1083	7.5
Modified soda	65	0.05	1300	6.2

Table IV is practically self explanatory. The alkali concentration given is that for maximum efficiency. The alkali efficiency index is obtained by dividing the arbitrary efficiency value of 65 by the alkali concentration. Hence the alkali efficiency indices compare the relative efficiencies per unit weight of builder. This is expressed in a different manner in column No. 5 where pound efficiency indices are given. The number of pounds of other builders that will do the same work as one pound of caustic soda are listed. In order of decreasing efficiency they are caustic soda, soda ash, modified soda, sodium metasilicate, and trisodium phosphate.

Another factor which must not be neglected is the amount of soap to be used with each of these builders to produce adequate lathering. In Table V the amount of soap used in washing 225-lb. loads of very dirty white work in a 42 by 84 in. wash wheel using a 5-in. water level is given. A high suds was maintained on the break operation and a good running suds on the following operations.

From Table V it is readily seen that caustic soda particularly and also sodium metasilicate do not require as much soap as the other builders studied to produce good washing. This will further modify their efficiency indices given in Table IV.

Since the amount of soap used to wash a given quantity of soiled fabrics will vary, depending on

TABLE V
SOAP CONSUMPTION WITH VARIOUS ALKALIES

Alkali	Pounds of soap to wash 225 lb. of clothes
Caustic soda	2.6
Soda ash	4.0
Sodium metasilicate	3.4
Trisodium phosphate	4.0
Modified soda	4.2

local conditions, it is not proposed to advocate any definite soap allotment. However, some data on the quantities of alkali to use are very important. From the experience gained in the present work it is felt that a liquid soap stock to which alkali has been added is not in the interests of economy. It has been shown herein that definite concentrations of alkali produce a maximum detergent effect when sufficient soap is present to maintain a suds. Further additions of alkali are wasted. When alkali and soap are mixed in a built soap stock one cannot be used without the other. Hence it is recommended to maintain a neutral liquid soap stock and add the builder either dry or in solution. In the case of caustic soda it is very essential that the alkali be added to the wash wheel in solution.

In Table VI the quantities of alkali found to produce optimum results in the present work are listed. Slight tolerances are allowable but must not exceed 2 or 3%. Sufficient soap was added to produce a high suds on the break and a running suds thereafter.

TABLE VI
ALKALI ADDITIONS FOR OPTIMUM RESULTS

Operation	Caustic soda, lb.	Soda ash, lb.	Sodium metasilicate, lb.	Trisodium phosphate, lb.	Modified soda, lb.
Break	0.10	0.30	0.62	0.72	0.60
Suds	0.09	0.25	0.55	0.60	0.55

Discussion of Results

Caustic Soda

In the present work caustic soda exhibited the highest detergent efficiency per unit weight. Several points in connection with its use cannot be too strongly stressed. Primarily the concentration range within which it exhibits its maximum effect is very narrow. Hence too great care cannot be taken in order that the proper amounts may be used. If these amounts are too great or too small faulty work will be the result. Care should be exercised in handling this substance. Solutions should always be prepared using cold water since much heat is evolved during the process of solution.

pH Values for Maximum Detergency

The pH values at which maximum detergent effects were noted varied considerably. For soda ash, trisodium phosphate, and modified soda the maximum pH values obtained during the sudsing operations were 10.1–10.2. Sodium metasilicate gave optimum results at a pH value of 10.8 and caustic soda at 10.6. These values are all lower than those obtained in the laboratory (2) and are at variance with the laboratory results of Rhodes and Bascom (3). The lowering of the pH values may be attributed to the residual acidity in the soiled fabrics as well as a slight hardness in the water used.

Free Rinsing Properties and Loading of the Fabrics

It is sufficient to say that no trouble was experienced in rinsing out any of the alkalis used in this work. Titration data combined with ash determinations on the fabrics after 20 washes conclusively proved this point. When a multiple rinse formula is used and an adequate supply of hot water is available no fears in this direction need be entertained.

Tensile Strength Losses

The test fabrics used in these experiments showed quite normal tensile strength losses after 20 washes in the majority of cases. The results are given in Table VII.

TABLE VII
TENSILE STRENGTH LOSSES

Alkali	Soda ash	Trisodium phosphate	Modified soda	Sodium metasilicate	Caustic soda
Tensile strength loss, %	10.7	8.0	8.5	12.2	11.4

The loss of 12.2% incurred with the use of sodium metasilicate is questionable. Other laundries using sodium metasilicate report tensile strength losses in the region of 10.5%. So many other factors blend to give the tensile strength loss that the builder alone cannot be held responsible.

Efficiency Indices

The efficiency indices of the alkalis calculated in Table IV agree remarkably well with those obtained in previous laboratory tests (2). The same order of efficiency was obtained. In the laboratory tests modified soda was not included. This product is third in the order of decreasing efficiency.

Acknowledgments

The writer wishes to express his appreciation to Mr. H. G. Vail and staff of Vail's Laundry, Ottawa, for their assistance in these tests; to Mr. B. J. Kenalty for his supervision of the power laundry tests; and to Mr. C. W. Davis of these laboratories for his analyses of the alkalis used.

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THE ALKALOIDS OF FUMARACEOUS PLANTS

VII. *Dicentra eximia* (KER) TORR.¹BY R. H. F. MANSKE²

Abstract

A chemical examination of *Dicentra eximia* has resulted in the isolation of six alkaloids. Protopine and dicentrine together constitute the greater portion of the total bases while *d*-glaucine was obtained in minute amounts. Two of the three other alkaloids, all of which appear to be new, resemble corydine and isocorydine and have been named *eximine* and *eximidine* respectively. They are isomeric and are best represented by the formula, $C_{17}H_{18}N(OMe)_2(OH)$. The sixth base was obtained in traces only and until further characterized will be referred to as alkaloid (δ). The yields of purified alkaloids obtained were as follows: dicentrine, 0.41; glaucine, 0.03; *eximine*, 0.30; *eximidine*, 0.04; protopine, 0.45; alkaloid (δ), 0.01%; total, 1.24%.

The alkaloid isolated by Eggleston, Black and Kelly from *D. eximia* and named *eximine* by them is identical with dicentrine.

Only three species of *Dicentra* are native to eastern North America and of these *D. canadensis* and *D. cucullaria* formed the subject of the first two papers of this series (4, 5). In continuation of a program of research previously outlined and in order to render the investigations sufficiently comprehensive it was deemed essential to include the third representative, namely, *D. eximia*. This attractive plant is native to the eastern United States growing quite abundantly in southwestern Virginia from which locality the greater part of the material used in the present investigation was obtained. In Virginia and probably elsewhere it is known by the appropriate name of wild bleeding heart. It has been introduced into cultivation for ornamental purposes and is adequately described botanically by Eggleston, Black, and Kelly (1).

The only chemical examination on record is one by the above authors (1), who reported the isolation of a crystalline base, *eximine*, melting at 165° C. (? corr.) together with amorphous bases. *Eximine* was stated to yield a hydrochloride of exceptionally slight solubility in cold water—a property which rendered its isolation particularly facile, and no difficulty was encountered in isolating the same base in the course of the present investigation.

Aside from this base, protopine and *d*-glaucine together with three new bases, all phenolic or weakly acidic, were obtained in the present work.

The *eximine* of Eggleston and coworkers was found to melt at 169° C.* and yielded on analysis figures which point unmistakably to the empirical formula $C_{20}H_{21}O_4N$. The methiodide and the derived methine base are normal and confirm the formula. Phenolic hydroxyls are absent and a Zeisel determination indicates the presence of two methoxyl groups. Finally, Hoffmann degradation yields trimethylamine in two stages so that an N-methyl group is functional.

¹ Manuscript received March 28, 1933.

Contribution from the National Research Laboratories, Ottawa, Canada.

² Associate Research Chemist, National Research Laboratories, Ottawa.

* All melting points are corrected.

Dicentrine is the only known alkaloid which possesses all of these properties. On some points, however, the literature is either vague or incomplete and for the opportunity of an exact comparison the author is greatly indebted to Dr. R. D. Haworth, formerly of Oxford University, who supplied a liberal specimen of authentic *d*-dicentrine. Although the latter had discolored slightly it still melted at 165° C. and admixture with the base from *D. eximia* showed no depression. The picrates from both sources, either alone or admixed, melted at 180° C. Since a remote possibility of non-identity still existed further comparison seemed desirable, and no derivative more suitable than the methine base could have been chosen. Not only is it obtainable in quantitative yield, but it crystallizes with great facility. Alone or admixed, the methines from both sources melted at 159° C. The name *eximine* for this base is therefore to be deleted from the literature.

In view of the great morphological similarity of *D. eximia* and *D. formosa* it is not surprising that dicentrine should be a common constituent.

The isolation of the remaining alkaloids, adequate details of which are given in the experimental section, was accomplished by a procedure which was partly developed in the case of *D. canadensis* (4) and somewhat elaborated and more specifically described in a communication treating of *Adlumia fungosa* (6). Well over 95% of the total basic fraction has been obtained in pure crystalline form, and efforts to isolate pure individuals from the small residuum are in progress.

Protopine, that universal constituent of Fumaraceous plants, was obtained in considerable quantity and in a high degree of purity.

Associated with dicentrine in the non-phenolic bases the hydrochlorides of which are removed from aqueous solution by means of chloroform (Fraction —BC) (6) a small amount of *d*-glaucine was found. It is pertinent to note that the latter has been isolated hitherto only from *Glaucium luteum* (*flavum*).^{*} Glaucine differs from dicentrine only by the substitution of two methoxyls for one methylenedioxy group, and it is of interest to learn of another example of a plant which is capable of effecting the two types of etherification on the one phenolic precursor. The relative proportion of the two types of ethers is perhaps an indication that methylenation is the more facile of the two processes when one of the hydroxyls is in a sterically hindered position. The widespread occurrence of protopine and the occasional though meagre co-presence of cryptopine may be regarded as a similar case.

One of the three presumably new alkaloids was obtained in excessively minute amounts. It has been obtained in a crystalline condition only in the form of salts, and further characterization has been delayed until more material is available. It resembles bicucine (7), in that it is soluble in an excess of aqueous ammonia as well as in fixed alkali, but does not appear to be identical with it. It will be referred to as alkaloid δ .

The remaining two alkaloids to which it is proposed to assign the names *eximine†* and *eximidine* are phenolic and both are removable from alkaline

^{*}The *laevo*-form has recently been obtained from *Corydalis ternata* (3).

[†]This term is again available for a new alkaloid in view of the fact that the alkaloid formerly described by this name (*vide supra*) has been identified with dicentrine.

solution by means of ether. In this respect they resemble corydine and isocorydine, with which pair they are isomeric, analyses of each yielding figures which are in substantial agreement with the empirical formula, $C_{20}H_{25}O_4N$. The analogy with corydine and isocorydine extends to the presence of one hydroxyl and three methoxyl groups, experiments on the allocation of which are in progress, the assumption being that both alkaloids are aporphine bases.

Separation of eximine and eximidine was effected by fractional crystallization of the hydrobromides from water, eximine hydrobromide being only very sparingly soluble. Eximine, like corydine, crystallizes with methanol of crystallization, which is retained very tenaciously. The crystals obtained by adding ether to a concentrated methanolic solution retain one molecule of the latter solvent and then melt at 117°C . When recrystallized from pure ethyl acetate no solvent of crystallization is retained and the alkaloid then melts at 142°C .

Eximidine was obtained in lesser amounts than its isomer. It melts at 133°C . and does not appear to retain solvent of crystallization with any tenacity.

The above record applies only to the roots and underground portion of the plant. The investigation of the aerial portion is in progress.

Experimental

The designations used in the following section refer to the fractions obtained in a manner precisely analogous to the procedure outlined in the case of *Adlumia fungosa* (6).

Isolation of Dicentrine

The methanolic extract (E) after removal of solvent, yielded after two extractions with dilute hydrochloric acid the residue (R) and the aqueous solution (S). Owing to the very sparing solubility of dicentrine hydrochloride in cold water the dark residue (R) contained a large amount of this salt. It was partly removed by repeated extraction with hot water and filtration through a layer of charcoal. The combined extracts were evaporated somewhat and the hydrochloride which separated on cooling was filtered off. The filtrate was added to (S).

The insoluble residue retained dicentrine very tenaciously and to recover the remainder, the mixture which had become contaminated with charcoal was exhausted in a Soxhlet apparatus first with petroleum ether (which extract proved to be excessively rich in sterols), then with ether (which extract is in process of examination), and finally with methanol. The last extract was poured into boiling dilute hydrochloric acid and the insoluble residue (the examination of which yielded nothing definite) filtered off. The filtrate on cooling deposited a copious yield of dicentrine hydrochloride, the final filtrate from which was combined with (S). To the latter solution there was also added the mother liquor from a recrystallization of all of the dicentrine hydrochloride obtained as described above.

The solution (S) was clarified by means of a little charcoal and exhausted with chloroform (C). The non-phenolic base (BC) obtained from this was converted to hydrochloride and the dicentrine salt crystallized as completely as possible by working up the mother liquors.

There was thus obtained a second lot of dicentrine hydrochloride which was combined with the first, and the entire product converted into free base by treatment in hot aqueous solution with potassium hydroxide. The base which became granular and crystalline in several days was filtered off and washed with water. After drying at 65° C. it was dissolved in a large volume of methanol, filtered with the aid of charcoal, and evaporated somewhat. On cooling pale yellow stout prisms of dicentrine, melting at 168° C. were obtained. After two more recrystallizations from the same solvent the crystals were brilliantly colorless and melted constantly at 169° C. When admixed with an authentic specimen of *d*-dicentrine melting at 165° the melting point was not quite sharp at about 167° C. Calcd. for $C_{20}H_{21}O_4N$: C, 70.80; H, 6.20; N, 4.13; 2 OMe, 18.20%. Found: C, 70.75; H, 6.27; N, 4.38; OMe, 18.45%.

The picrates of both specimens were prepared side by side in hot methanolic solution. After one recrystallization, from the same solvent and washing with ether, the picrates melted at 180° C. either alone or admixed.

Dicentrine Methine

The methiodide of dicentrine when recrystallized from water is stated to retain a molecule of water of crystallization and then melt at 224° C. It has now been found that it is more conveniently recrystallized from methanol, particularly if a little ether is added, and then retains one molecule of methanol of crystallization. As thus obtained the substance melts at 188-189° C. Calcd. for $C_{20}H_{21}O_4N \cdot CH_3I \cdot CH_3OH$; N, 2.73; I, 24.75%. Found: N, 2.87; I, 24.06%.

For the preparation of the methine base it is not necessary or desirable to purify the methiodide. Furthermore, dicentrine of almost any quality as long as it is crystalline will suffice. The free base is dissolved in a mixture of chloroform and methanol and heated under reflux for about one hour with an excess of methyl iodide. The solvent is then distilled off, the residue dissolved in hot water, filtered from a slight turbidity and the cooled filtrate basified with an excess of potassium hydroxide. When impure dicentrine has been used, a turbidity may be produced at this point. In order to remove this, and as a general precaution it is desirable to filter the alkaline solution again with the aid of charcoal. The brilliantly clear and generally colorless filtrate is then heated on a steam bath. In a short while a slight turbidity develops, and this is shortly replaced by brilliant colorless flat plates which are filtered off after several hours of heating. As thus prepared the thoroughly washed and dried *dicentrine methine* melts sharply at 158-159° C. When recrystallized from methanol in which it is only sparingly soluble when cold it melts at 159° C. A dilute alcoholic solution displays a very intense bluish-violet fluorescence.

The methine prepared from a specimen of *d*-dicentrine supplied by Dr. R. D. Haworth exhibited identical properties. Calcd. for $C_{21}H_{23}O_4N$; C, 71.39; H, 6.52%. Found: C, 71.61; H, 6.94%.

Dicentrine methine hydrochloride was recrystallized from hot water and then consisted of colorless needles which are only sparingly soluble in cold water or methanol. When heated in a melting point tube there is no appreciable color change until a temperature of 302° C. is reached when melting and effervescence take place.

The methine methiodide was prepared by treatment with an excess of methyl iodide in methanol-chloroform. After removal of the solvents the residue was recrystallized from a large volume of hot water in which it is only sparingly soluble. It was obtained in very pale yellow fine prisms. When heated with excess potassium hydroxide in aqueous solution, trimethylamine is given off and a partly crystalline deposit is obtained. This polymerized somewhat when attempts to recrystallize it were made. It is evidently a substituted phenanthryl-ethylene.

Isolation of Glaucine

The aqueous mother liquor from the dicentrine hydrochloride obtained from (BC) (p. 595) was basified with excess potassium hydroxide, and the liberated base extracted with a large volume of ether. The ether solution was repeatedly washed, first with aqueous potassium hydroxide and then with water. It was then dried over potassium carbonate and the solvent partly distilled off. The concentrated solution deposited a small amount of protopine in the course of several days. This was removed and the base in the mother liquor converted to hydrochloride again. In the course of several days a small amount of dicentrine hydrochloride crystallized out. This was filtered off and the base again regenerated from the mother liquor. Extraction of the basified solution with ether as before and evaporation of the solvent yielded a pale yellow resin which in this condition failed to crystallize. It was therefore completely freed of ether and the residue repeatedly extracted with boiling petroleum ether (50-60° C.). An insignificant amount of dark tarry insoluble material was discarded. The combined extract was evaporated to a small volume when a further small amount of dark resin separated. This was again removed and the resulting pale yellow solution evaporated until a second viscous liquid phase separated. Sufficient highly purified ether to redissolve the latter was added and the solution set aside in a warm place in a stoppered flask. In the course of several days a crust of pale yellow crystals had separated. After removal of the mother liquor the solid (m.p., 117-119° C.) was washed cautiously with ether and recrystallized from the same solvent. As thus obtained the *glaucine* consisted of very pale yellow prisms melting sharply at 120° C. It dissolved in cold sulphuric acid to a colorless solution which slowly became blue. When the solution was heated an intense violet or purple coloration was developed which changed to orange on dilution with water. Calcd. for $C_{21}H_{23}O_4N$; C, 70.99; H, 7.04; 4 OMe, 34.93%.

Found: C, 71.17; H, 7.11; OMe, 34.76%. The base was further identified as glaucine by comparison with an authentic specimen which, after recrystallization from hexane, melted sharply at 120° C. A mixture of the bases from the two sources melted at the same temperature. The author is greatly indebted to Prof. G. Barger of the University of Edinburgh for the specimen of glaucine which was prepared from boldine.

The non-phenolic aporphines are characterized by the readiness with which they react with alkyl chloro-formates in the presence of alkali to yield N-carbethoxy derivatives of β -methylaminoethyl-phenanthrenes (2, 8). The latter are for the greater part well crystallized compounds and may serve for the purpose of characterization.

Since the substance from glaucine does not appear to have been described its preparation was effected by the method of Osada (8).

N-Carbethoxy-8-(β -methylaminoethyl)-2, 3, 5, 6-tetramethoxy-phanthrene was obtained in large colorless rhombic plates sparingly soluble in cold methanol or ether, but very soluble in chloroform; m.p., 115° C. Calcd. for $C_{20}H_{25}O_6N$; N, 3.28%. Found: N, 3.27%.

Isolation of Eximine

The fraction (EC) (namely, the bases whose hydrochlorides are extracted from an acid aqueous solution with chloroform and which are removed from solution in excess fixed alkali by means of ether) was treated in methanol with an excess of concentrated hydrobromic acid. Crystallization was almost immediate and practically complete. The hydrobromides were filtered off, washed with cold methanol and recrystallized twice from hot water. The least soluble fraction then had the appearance of homogeneity.

Eximine hydrobromide as thus obtained consists of colorless equilateral deep rhombic plates which when rapidly heated begin to darken at 248° C. and melt with decomposition at 270° C.

The free base is conveniently obtained from the hydrobromide as follows. The salt is dissolved in hot water and the clear solution cautiously treated with dilute ammonia solution until the incipient turbidity is just permanent. In a short time the free base crystallizes and the addition of ammonia is then cautiously continued until an excess is present. The mixture is allowed to remain overnight in an ice chest and the crystals filtered off, and washed with cold water. After drying in an oven at 65° C. the base as thus obtained melts at 142-143° C.

Eximine is best recrystallized from purified ethyl acetate, in which it is moderately soluble, and then consists of stout quadrangular prisms with oblique terminations. When this product is thoroughly dried it melts at 142° C. Calcd. for $C_{20}H_{23}O_4N$; C, 70.36; H, 6.75; N, 4.13; 3 OMe, 27.27%. Found: C, 69.58; H, 6.95; N, 4.48; OMe, 27.25%.

Eximine may also be readily recrystallized by adding dry ether to a concentrated methanolic solution. Long stout needles are thus obtained which melt not quite sharply at 116-117° C. with the evolution of a gas, probably

methanol.⁴ When this substance was dried overnight in a high vacuum over phosphorus pentoxide and then dried to constant weight at 100° C. it lost 5.5% of its weight. It is therefore probable that in the freshly crystallized substance one molecule of methanol (8.6%) is present, part of which was lost in the high vacuum. This behavior is strictly analogous to that of corydine.

Eximine methiodide was prepared by treating a chloroform-methanol solution of the base with excess methyl iodide. After 24 hr. the solvent was removed, the residue dissolved in water and the filtered solution (charcoal) evaporated to a syrup. The addition of a little methanol and then ether yielded colorless elongated prisms sparingly soluble in cold methanol. When heated, solvent of crystallization was lost at about 180° C. at which temperature slight darkening took place. At 212° C. melting with charring and effervescence took place. Analyses are in agreement with the dihydrate. Calcd. for $C_{20}H_{23}O_4N \cdot CH_3I \cdot 2H_2O$; N, 2.70; I, 24.47%. Found: N, 2.70; I, 24.95%.

Isolation of Eximidine

The methanolic mother liquor from the first crystallization of *eximine* hydrobromide was freed of solvent and the residue dissolved in hot water. Excess potassium hydroxide was added to the filtered solution (charcoal). A small amount of protopine was precipitated. The filtrate from this was saturated with ammonium chloride and extracted with ether. The extract was evaporated to a small volume and a turbidity removed by filtration with charcoal. The filtrate on further evaporation rapidly deposited fine colorless needles which were filtered off and washed with cold ether in which the base is sparingly soluble. It was recrystallized by dissolving in a little acetone, evaporating to a resin and adding a large volume of ether. A slight turbidity was removed with the aid of charcoal and the filtrate rapidly evaporated. Long colorless needles of *eximidine* were thus obtained which melted sharply at 133° C. Calcd. for $C_{20}H_{23}O_4N$; C, 70.36; H, 6.75; N, 4.13; 3 OMe, 27.27%. Found: C, 69.42; H, 6.73; N, 4.26; OMe, 27.00%.

Eximidine methiodide prepared in the usual way proved to be very difficult to purify. After many charcoal treatments a methanol solution was treated with ether and the turbidity rapidly filtered off. The filtrate then deposited colorless slender needles which after filtering off were washed with ethyl acetate and with ether. They began to darken at 190-195° C. and frothed to a black mass at 218° C. Calcd. for $C_{20}H_{23}O_4N \cdot CH_3I$; I, 26.29%. Found: I, 25.64%.

Isolation of Protopine

In the writer's experience the plant under discussion offers one of the most convenient sources of protopine. The fraction (AC) when partly freed of chloroform and treated with hot methanol yielded a large quantity of the base which in this condition was only pale brown and melted at 208-209° C.

The mother liquor on appropriate treatment yielded the non-phenolic fraction (BS) which on recrystallization from chloroform-methanol proved to consist almost entirely of pure protopine. The alkaloid was obtained in the

two crystal forms (6) both of which were identical with those from *Adlumia fungosa* and from other sources. No difficulty was encountered in raising the melting point to 211° C. by one or two recrystallizations.

The final mother liquors from the crystallization of the protopine were converted to hydrochlorides. A further amount of protopine as hydrochloride was thus obtained. The mother liquor from this yielded only a few milligrams of amorphous base.

Isolation of Alkaloid δ

The fractions (ES, BSE, and EES) were combined, dissolved in dilute hydrochloric acid and the filtered solution basified with excess potassium hydroxide. A small amount of protopine was precipitated and a further small amount was obtained by extracting the alkaline solution with ether. The alkaline solution was then saturated with carbon dioxide and the precipitated base filtered off and dried. Treatment in methanol with concentrated hydrobromic acid and cautious addition of ether yielded a pale yellow hydrobromide which was recrystallized from methanol by the addition of ether. It was thus obtained in almost colorless fine needles sparingly soluble in cold water. It darkens at 235° C. and melts to a black mass at 239° C. The hydrochloride similarly prepared began to darken at 234° C. and decomposed to a black tar at 237-238° C.

Cautious treatment of an acid solution with ammonia first yields a flocculent precipitate which dissolves in an excess of the reagent.

When a solution of the base in excess of ammonia is freed of the latter in a desiccator over sulphuric acid a semicrystalline crust is obtained. This product is readily soluble in methanol but attempts to obtain the free base in a pure state have thus far failed.

Acknowledgment

The author wishes to express his indebtedness to Dr. Helen Stantiel of Toronto University who kindly submitted some of the analytical figures.

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β -ANTHRONYL- β -PHENYL-PROPIONIC ACID AND ITS DERIVATIVES

SYNTHESIS OF THE γ -ANTHRONYL- α -HYDRINDONE¹

BY PAUL E. GAGNON² AND LUCIEN GRAVEL³

Abstract

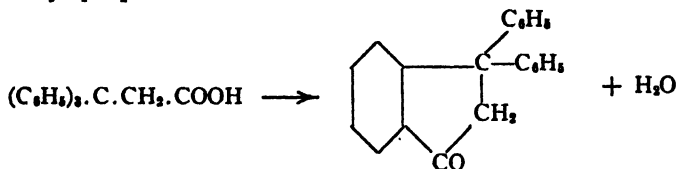
Benzaldehyde and *o*-chlorobenzaldehyde were readily condensed with ethyl and methyl malonate, giving rise to unsaturated esters. The esters were condensed with anthrone in alcoholic solution in the presence of piperidine. Hydrolysis of the product with sulphuric acid yielded β -anthronyl- β -phenyl-propionic acid. The chloride of this acid was formed by the action of phosphorus pentachloride. Two tautomeric derivatives of γ -anthronyl- α -hydrindone result from the action of aluminium chloride on β -anthronyl- β -phenyl-propionyl chloride. No benzanthrone derivatives were formed.

The action of bromine on the two tautomeric forms of γ -anthronyl- α -hydrindone gave rise to one monobromide only, and oxidation of the two tautomers yielded only anthraquinone and phthalic acid.

The method of preparation of β -anthronyl- β -phenylpropionic acid has been improved and the following compounds have been described, as far as the authors are aware, for the first time: the dimethyl and diethyl esters of *o*-chlorbenzylidene-malonic acid and anthrone-*o*-chlorbenzylidene-malonic acid; anthrone-benzylidene-diethyl-malonate; β -anthronyl- β -(*o*-chlorphenyl)-propionic acid; the silver salt, chloride, amide, anilide, methyl and ethyl esters of β -anthronyl- β -phenyl-propionic acid; γ -anthronyl- α -hydrindone (enolic and ketonic forms); γ -anthronyl- β -brom- α -hydrindone; and the hydrazone hydrate, phenylhydrazone, oxime and semicarbazone of γ -anthronyl- α -hydrindone.

Introduction

In recent investigations, one of the writers (2) showed that γ - γ -diphenyl- α -hydrindone (13, p. 1370) can be prepared with good yields by cyclisation of triphenyl-propionic acid.



It was thought interesting to see whether β -anthronyl- β -phenyl-propionic acid, prepared by Meerwein (10, p. 285), would react in the same way and give rise to a new derivative of α -hydrindone (II), or whether, by reacting differently, it would give rise to a derivative of benzanthrone (I).

The main object of the present work was the preparation of β -anthronyl-3-phenyl-propionyl chloride and the study of its cyclisation.

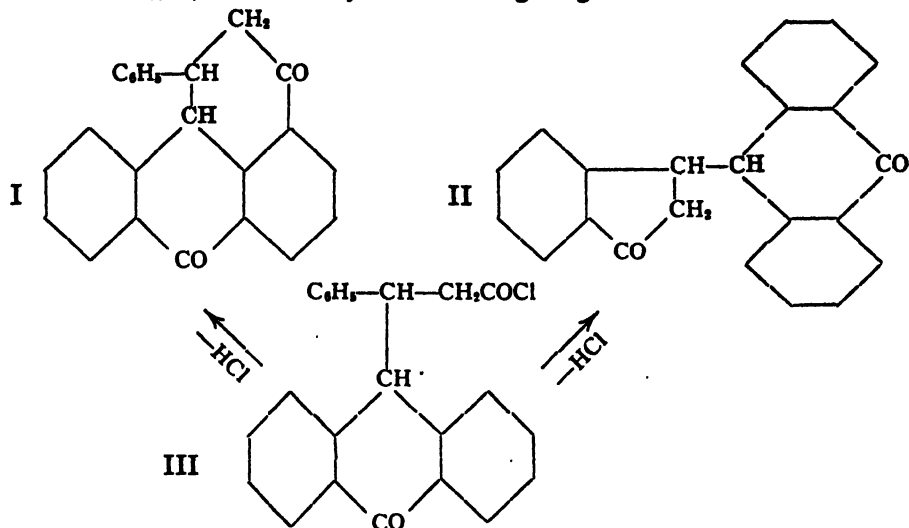
¹ Manuscript received March 27, 1933.

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Theoretically *β*-anthronyl-*β*-phenyl-propionyl chloride (III), by elimination of one molecule of hydrochloric acid, can form either of the two isomeric ketones $C_{22}H_{16}O_2$, as shown by the following diagram:



Only one of these compounds, (I), was mentioned in the literature; its enolic form has been obtained by elimination of water from *β*-anthronyl-*β*-phenyl-propionic acid with sulphuric acid (4, 5, 6).

The writers were not able to obtain it. On the other hand, they did obtain *γ*-anthronyl-*α*-hydrindone (II), the two tautomeric forms of which have not hitherto been described. One of the forms melts at $161-162^\circ C.$ giving a monobromide melting at $170-171^\circ C.$; the other melts at $187-188^\circ C.$ and gives the same monobromide.

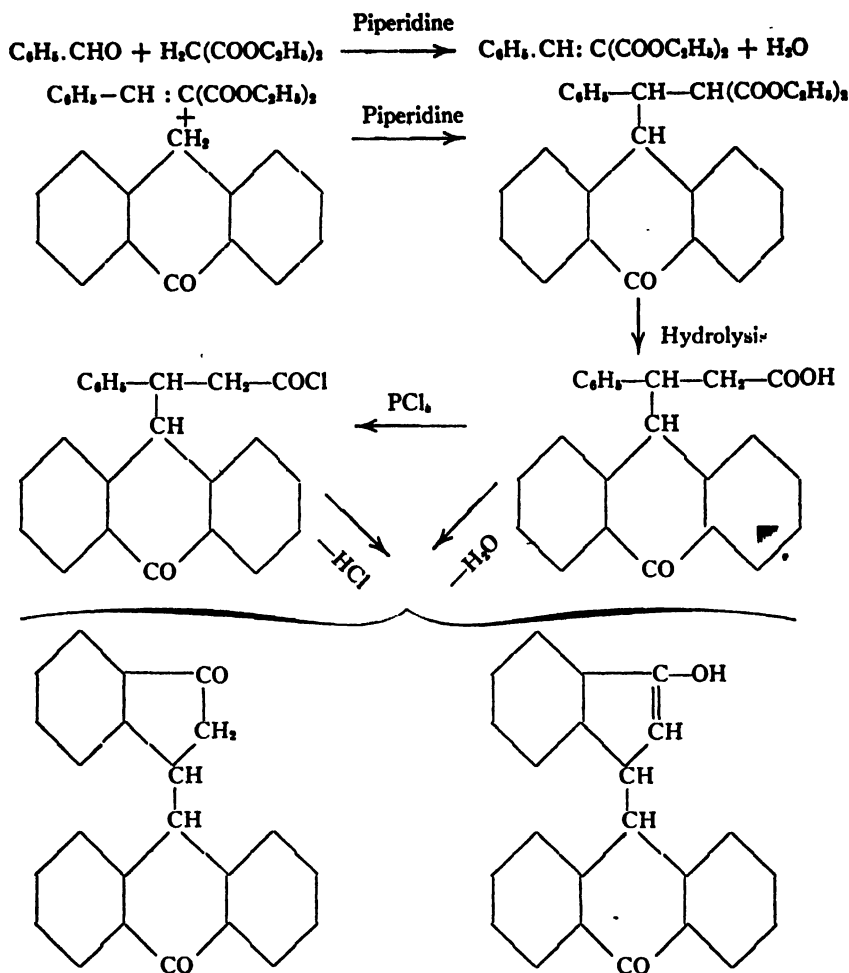
The writers have prepared the two tautomeric forms, by the action of aluminium chloride on the chloride of *β*-anthronyl-*β*-phenyl-propionic acid and also from the acid itself by elimination of water with sulphuric acid.

The synthesis was carried out in accordance with the reactions shown on p. 602.

Instead of benzylidene-dimethyl-malonate, used by Meerwein (10, p. 285), benzylidene-diethyl-malonate (7) which is cheaper was employed as the starting material.

Benzylidene-diethyl-malonate was condensed with anthrone and anthrone-benzylidene-diethyl-malonate obtained. By hydrolysis of this ester with sulphuric acid, *β*-anthronyl-*β*-phenyl-propionic acid was formed. By treating this acid with phosphorus pentachloride, the chloride was obtained in very good yields. It is a white substance melting at $115-116^\circ C.$ and not very stable. It was, however, well characterized by preparing many derivatives, and used as starting material for the preparation of *γ*-anthronyl-*α*-hydrindone.

In order to establish the constitution of this ketone, the writers determined its molecular weight, and oxidized it by nitric acid according to König's method (8). The only resulting products are anthraquinone and phthalic acid.



This paper deals with: (a) the various methods used to obtain β -anthronyl- β -phenyl-propionic acid and β -anthronyl- β -(*o*-chlorphenyl)-propionic acid; (b) a study of β -anthronyl- β -phenyl-propionyl chloride and some of its derivatives; and (c) a description of the methods of preparation of γ -anthronyl- α -hydrindone, some of its derivatives, and its oxidation products.

Part I

β -ANTHRONYL- β -PHENYL-PROPIONIC ACID AND β -ANTHRONYL- β -(*o*-CHLORPHENYL)-PROPIONIC ACID

In this section will be considered: (a) the unsaturated esters, benzylidene-diethyl-malonate, and the diethyl and dimethyl esters of *o*-chlorbenzylidene-malonic acid; (b) their condensation products with anthrone; and (c) the methods used for obtaining β -anthronyl- β -phenyl- and β -anthronyl- β -(*o*-chlorphenyl)-propionic acids.

I. Unsaturated Esters

1. *Benzylidene-diethyl-malonate*.—Benzylidene-diethyl-malonate ($C_{16}H_{18}.CH : C(COOC_2H_5)_2$) is readily obtained by Knoevenagel's method (7, p. 2591), by condensation of equivalent quantities of benzaldehyde and diethyl-malonate. From 1200 gm. of diethyl-malonate there was obtained 1660 gm. of benzylidene-diethyl-malonate distilling at 184-184.5° C./9 mm. and at 176-177° C./5 mm. Yield, 80%.

2. *o*-Chlorbenzylidene-diethyl-malonate.—*o*-Chlorbenzaldehyde in the presence of piperidine was condensed with diethyl-malonate in a manner similar to benzaldehyde.

The ester formed in this reaction possesses properties very similar to those of benzylidene-diethyl-malonate. It is easily purified, as it distils without decomposition under reduced pressure.

Preparation.—In a 200-cc. conical flask, 50 gm. of diethyl-malonate and 48.75 gm. of *o*-chlorbenzaldehyde were introduced. The mixture was stirred and treated with two grams of piperidine. The well-corked flask was then left at room temperature for 4-5 days. The water formed during the condensation was evaporated off on the water bath.

After cooling, the mixture was extracted with ether and the ethereal solution, washed many times with dilute hydrochloric acid and then with water, was dried over anhydrous sodium sulphate. Ether was distilled off and the sirupy liquid remaining distilled under reduced pressure. Between 178-179° C./4 mm. the distillate was colorless; b.p., 181-182° C./5 mm. The ester was still impure, containing *o*-chlorbenzoic acid which crystallized in small white needles when the distillate was cooled to 0° C. This acid was formed by the oxidation of *o*-chlorbenzaldehyde during the distillation.

To obtain the pure ester the distillate was extracted with ether, the ethereal solution treated with concentrated ammonia, washed many times with water and dried over anhydrous sodium sulphate. The ether was distilled off, and the resulting product redistilled under reduced pressure. The ester then distilled at 182-183° C./5 mm. Yield, 59.6 gm. (theory, 87.7 gm.), i.e., 68%.

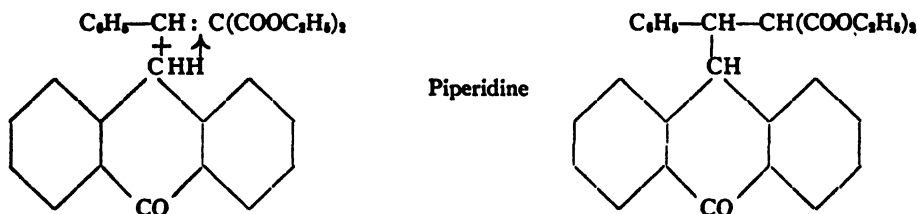
The ester is a colorless liquid having a characteristic odor. On standing it crystallizes, forming colorless prisms; m.p., about 30° C. Analysis:—Calcd. for $C_{14}H_{16}O_4Cl$; Cl, 12.53%. Found: Cl, 12.78, 12.54%.

3. *o*-Chlorbenzylidene-dimethyl-malonate.—Dimethyl-malonate was condensed with *o*-chlorbenzaldehyde as readily as diethyl malonate and the method used was the same. Fifty grams of dimethyl-malonate, 55 gm. of *o*-chlorbenzaldehyde and 2 gm. of piperidine were used. A colorless product was obtained: b.p., 175° C./3 mm. It crystallized on stirring. Yield, 60.6 gm. (63%). The ester is very soluble in most organic solvents, crystallizes readily from petroleum ether and is a colorless substance; m.p. 58-59° C. Analysis: Calcd. for $C_{12}H_{14}O_4Cl$; Cl, 13.93%. Found: Cl, 13.62%.

II. Products of Condensation of the Unsaturated Esters with Anthrone

The above-mentioned esters can be condensed with anthrone in the presence of piperidine to form addition products similar to anthrone-benzylidene-dimethyl-malonate prepared by Meerwein (10, p. 285).

The following reaction takes place:—



Anthrone has been prepared by reduction of anthraquinone, dissolved in acetic acid, by means of tin and hydrochloric acid (1, 11, p. 55, 12, p. 8). The anthrone must be free from acetic acid. It was therefore treated with sodium bicarbonate and recrystallized from benzene.

The method of condensation was the same for the three esters.

1. *Anthrone-benzylidene-diethyl-malonate* $\text{C}_6\text{H}_5.\text{CH}(\text{C}_{14}\text{H}_9\text{O}).\text{CH}(\text{COOC}_2\text{H}_5)_2$. This ester was made and used as the starting material for the preparation of β -anthronyl- β -phenyl-propionic acid.

Anthrone (31.3 gm.), crystallized from benzene and free from acetic acid, 43 gm. of benzylidene-diethyl-malonate, 80 cc. of anhydrous methyl alcohol and 30 drops of piperidine were introduced into a 300-cc. flask. A condenser provided with a calcium chloride tube was attached and the flask heated on the water bath. It was very important to shake the flask, to hasten condensation. After 20 min., 20 additional drops of piperidine was added; 10 min. later the anthrone was completely dissolved and the solution had a reddish color. The flask was left on the water bath for another hour.

The solution was rapidly filtered with suction and cooled to 0° C. On stirring the solution, the product crystallized. It was filtered off, and washed with alcohol cooled to 0° C. There was thus obtained 64.5 gm. of a colorless substance melting at 103-104° C. Yield, 90.6%. Recrystallized from methyl alcohol, it melted at 104-105° C.

The ester is soluble in benzene, acetone, chloroform, methyl and ethyl alcohols, ether, and slightly soluble in petroleum ether. Sulphuric acid dissolves it, forming a yellowish solution. Analysis:—Calcd. for $\text{C}_{28}\text{H}_{26}\text{O}_6$: C, 76.00; H, 5.88%. Found: C, 75.96; H, 5.79%.

2. *Anthrone-o-chlorbenzylidene-diethyl-malonate*. Anthrone (27.44 gm.), o-chlorbenzylidene-diethyl-malonate (42 gm.), anhydrous methyl alcohol (25 cc.) and piperidine (25 drops) were introduced into a 300-cc. flask connected with a condenser. After heating on a water bath for an hour the anthrone dissolved. The warm solution was rapidly filtered and cooled; the product crystallizing out. It was filtered off, washed with alcohol and dried. Yield, 72.6%.

Anthrone-*o*-chlorbenzylidene-diethyl-malonate is very soluble in methyl alcohol, but is less soluble in ethyl alcohol. From these solvents it separates in colorless crystals; m.p., 119-120° C. Analysis:—Calcd. for $C_{22}H_{22}O_5Cl$: Cl, 7.43%. Found: Cl, 7.46, 7.57%.

3. *Anthrone-o-chlorbenzylidene-dimethyl-malonate*, $Cl.C_6H_4.CH(C_{14}H_9O)CH(COOCH_3)_2$. This substance was prepared in a manner similar to anthrone-*o*-chlorbenzylidene-diethyl-malonate. The reactants and amounts used were as follows: *o*-chlorbenzylidene-dimethyl-malonate, 21 gm.; anthrone, 15.2 gm.; anhydrous methyl alcohol, 41 cc.; piperidine, 30 drops. Yield, 29 gm. (82.6%). Recrystallized from benzene, which dissolves it readily, anthrone-*o*-chlorbenzylidene-dimethyl-malonate forms colorless prisms melting at 148-149° C. Analysis:—Calcd. for $C_{28}H_{21}O_5Cl$: Cl, 7.93%. Found: Cl, 7.96, 7.97%.

III. *β*-Anthronyl-*β*-phenyl-propionic Acid and *β*-Anthronyl-*β*-(*o*-chlorphenyl)-propionic Acid

These two acids were prepared by hydrolysis of the malonates just described. The reaction was complete only after heating for four or five days. The speed of the reaction was increased by adding to the hydrolyzing mixture, after boiling for two days, a few crystals of the acid. This caused crystallization of the acid already formed and facilitated the hydrolysis of the remaining ester.

1. *β*-Anthronyl-*β*-phenyl-propionic acid $C_6H_5.CH(C_{14}H_9O)CH_2COOH$. Two methods of preparation of this acid are known. It was first obtained by Meerwein (10), and more recently (4, 5) by condensation of cinnamic acid with anthrone. The writers used Meerwein's method, employing the diethyl ester instead of the dimethyl ester as the starting material.

Preparation.—Anthrone-benzylidene-diethyl-malonate (210 gm.), 30% sulphuric acid (1500 cc.) and glacial acetic acid (600 cc.) were introduced into a three-litre flask fitted with a rubber stopper and a reflux condenser, and the mixture heated to boiling. The ester melted and separated into small drops which were well stirred by boiling the hydrolyzing mixture. After heating for five days, all the droplets solidified. However, on comparison with a sample of *β*-anthronyl-*β*-phenyl-propionic acid, it was observed that the hydrolysis was complete on the fourth day. On seeding, the acid crystallized almost completely; the following day, all the acid had solidified. The contents of the flask was then poured into an equal volume of water. The acid was filtered off, pulverized, washed with water, filtered off and dissolved in a hot saturated solution of sodium bicarbonate. After cooling, the solution of the sodium salt was filtered and acidified with sulphuric acid. The precipitated acid was filtered off and washed with water until free from sulphuric acid. Yield, 135 gm. (82%). The acid obtained by recrystallization from acetic acid and washing with alcohol was white; m.p., 196-197° C. Yield, 102.3 gm. (63%). The residual mother liquor deposited about 9-10 gm. of rather impure acid.

When heated at 100°C. with concentrated sulphuric acid, the acid remains unaltered. It may be distilled under reduced pressure without decomposition. This acid was identified as β -anthronyl- β -phenyl-propionic acid, prepared by Meerwein's method, by a mixed melting point determination.

It was characterized by its silver salt. A paste, made by mixing β -anthronyl- β -phenyl-propionic acid (1 gm., weighed exactly) with a small quantity of water, was dissolved in a few drops of concentrated ammonia and filtered. An excess (0.2-0.4 cc.) of a *N*/10 silver nitrate solution was added to the filtrate with vigorous stirring. The insoluble silver salt separated out immediately in white flakes and was allowed to settle in the dark. The precipitate, washed twice with hot water by decantation, was filtered off, washed again with hot water and dried in the dark over sulphuric acid. Analysis:—Calcd. for $C_{23}H_{17}O_2Ag$: Ag, 24.02%. Found: Ag, 23.91, 23.89%.

2. β -Anthronyl- β -(*o*-chlorphenyl)-propionic acid $Cl.C_6H_4.CH(C_{14}H_9O)CH_2.COOH$.

(a) *Preparation from anthrone-*o*-chlorbenzylidene-diethyl-malonate.* Anthrone-*o*-chlorbenzylidene-diethyl-malonate (4 gm.) was treated with 31 cc. of 30% sulphuric acid and 12 cc. of acetic acid. The flask was heated for five days at 130-135° C., and after cooling, the mixture was poured into 200 cc. of water. The insoluble acid was filtered off and dissolved in a hot solution of sodium bicarbonate. The solution was filtered and acidified with dilute sulphuric acid. The precipitated acid was filtered off, washed with water and dried; m.p. 155-156° C. Yield, 2.7 gm.

The acid crystallizes from acetic acid in very small diamond-shaped crystals melting at 159-161° C. It is very soluble in alcohol. Analysis:—Calcd. for $C_{23}H_{17}O_2Cl$: Cl, 9.41%. Found: Cl, 9.47, 9.45%.

(b) *Preparation from anthrone-*o*-chlorbenzylidene-dimethyl-malonate.* Anthrone-*o*-chlorbenzylidene-dimethyl-malonate (14.4 gm.) was treated with 40 cc. of acetic acid and 100 cc. of 30% sulphuric acid in a flask fitted with a rubber stopper and a reflux condenser. The mixture was boiled for six days, cooled and poured into water. The insoluble acid was filtered off, washed with water and dissolved in a hot saturated solution of sodium bicarbonate. From this solution, cooled and filtered, the acid was precipitated with dilute sulphuric acid, filtered off and dried at 80° C. Yield, 10.4 gm. M.p. 120° C.

The raw product was easily purified by crystallizing it from acetic acid and washing with ether; m.p. 159-161° C.

This acid was proved identical with that obtained by hydrolysis of anthrone-*o*-chlorbenzylidene-diethyl-malonate by a mixed melting point determination.

Part II

β -ANTHRONYL- β -PHENYL-PROPIONYL CHLORIDE AND ITS DERIVATIVES

In this section will be considered:—(a) the two methods for preparing the acid chloride; and (b) some derivatives of the acid prepared from the chloride, i.e., esters, amide, anilide, anthrone-benzylidene-acetophenone.

I. *β -Anthronyl- β -phenyl-propionyl-chloride* $C_6H_5.CH(C_{14}H_9O).CH_2.CO.Cl$. The chloride was first prepared with phosphorus trichloride but as resins were also formed, purification of the product was difficult. The pure chloride was obtained in good yields by the action of phosphorus pentachloride.

β -Anthronyl- β -phenyl-propionic acid (30 gm.), phosphorus pentachloride (24.5 gm.) and anhydrous carbon disulphide (180 cc.) were heated to boiling in a 300-cc. flask fitted with a reflux condenser and a calcium chloride tube. The insoluble acid gradually dissolved owing to the formation of the soluble chloride and there was a rapid evolution of hydrochloric acid. The mixture was boiled for about an hour.

After cooling, the solution was rapidly filtered, and the carbon disulphide and phosphorus oxychloride were partly distilled off. Upon cooling and stirring the remaining solution (50-60 cc.) the chloride crystallized; it was filtered off and dried in vacuum. Yield, 31 gm. It was purified by crystallization from anhydrous carbon disulphide. Yield, 28.6 gm. (90.4%). The pure chloride crystallizes from carbon disulphide in colorless diamond-shaped crystals melting at 115-116° C.

The chloride is not a very stable compound. By exposure to air it is slowly transformed into the acid, the same transformation being effected much more rapidly if the chloride is treated with acetic acid. A short boiling with acetic acid transforms it almost quantitatively. The chloride cannot be distilled without decomposition, for even under reduced pressure resins are formed. Analysis:—Calcd. for $C_{23}H_{17}O_2Cl$; Cl, 9.80; C, 76.54, H, 4.75%. Found: Cl, 9.91; C, 76.27; H, 4.67%.

II. *β -Anthronyl- β -phenyl-propionic Acid Derivatives Prepared from the Chloride*

1. *Methyl ester*, $C_6H_5.CH(C_{14}H_9O)CH_2.COOCH_3$. Pure β -anthronyl- β -phenyl-propionic acid (5 gm.), anhydrous benzene (50 cc.) and phosphorus trichloride (5 cc.) were heated together for two hours in a 200-cc. flask fitted with a reflux condenser and a calcium chloride tube. The solution was cooled to room temperature and 80 cc. of methyl alcohol was slowly added. A violent reaction took place, after which the mixture was boiled for 1½ hr. The hot solution was then rapidly filtered, the solvent distilled off and the ester crystallized. It was extracted with benzene, the solution washed with water to eliminate hydrochloric acid, dried with calcium chloride and filtered again; the solvent was partly distilled off and on cooling, the almost colorless ester crystallized.

It was filtered off, dried and recrystallized from methyl alcohol; m.p., 111-112° C. Yield, 4.4 gm. (84.6%). Analysis:—Calcd. for $C_{24}H_{20}O_2$: C, 80.86, H, 5.66%. Found: C, 80.68, 80.67; H, 5.58, 5.51%.

2. *Ethyl ester*, $C_6H_5.CH(C_{14}H_9O)CH_2.COOC_2H_5$. Pure β -anthronyl- β -phenyl-propionic acid (5 gm.), free from water, phosphorus trichloride (5 cc.) and anhydrous benzene (50 cc.) were boiled in a 250-cc. flask fitted with a reflux

condenser and a calcium chloride tube for $2\frac{1}{2}$ hr. After cooling, the solution was filtered and poured into a dry flask; 80 cc. of absolute alcohol was slowly added and the solution boiled for $1\frac{1}{2}$ hr. The solvent was distilled off.

A sirupy product which crystallized finally was left in the flask. The colorless ester was obtained by recrystallization from alcohol; m.p., 88-89° C. Yield, 3.7 gm. (69.5%). Analysis:—Calcd. for $C_{22}H_{22}O_2$: C, 81.04; H, 5.99%. Found: C, 80.93, 80.92; H, 6.00, 5.91%.

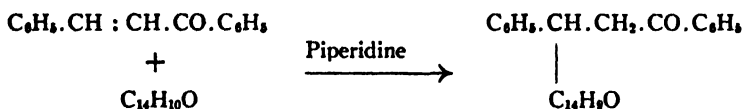
3. *Amide*, $C_6H_5.CH(C_{14}H_9O)CH_2.CO.NH_2$. A rapid stream of ammonia was passed through a solution of 7 gm. of β -anthronyl- β -phenyl-propionyl chloride in 25 cc. of anhydrous benzene. After two hours, the solvent had completely evaporated and a crystalline product remained in the flask. It was washed with water and dried at 80° C. Yield, 6.7 gm.

Recrystallized from methyl alcohol, the amide was colorless; m.p., 181-182° C. It is very soluble in benzene and alcohol, and insoluble in petroleum ether. Analysis:—Calcd. for $C_{22}H_{19}O_2N$: N, 4.10%. Found: N, 4.13, 4.10%.

4. *Anilide*, $C_6H_5.CH(C_{14}H_9O)CH_2.CO.NH.C_6H_5$. Two grams of β -anthronyl- β -phenyl-propionyl chloride was dissolved in 20 cc. of anhydrous benzene and 5 cc. of aniline added dropwise to the solution. The anilide immediately precipitated. It was filtered off and washed with water. The product was extracted with ether, and the ethereal solution washed with dilute hydrochloric acid and with water. Ether was distilled off and the remaining product dried. The anilide was purified by recrystallization from alcohol, from which it was deposited in very fine colorless needles. From ether the anilide crystallized in diamond-shaped crystals melting at 168-169° C. Analysis:—Calcd. for $C_{28}H_{23}O_2N$: N, 3.35%. Found: N, 3.36, 3.34%.

5. *Anthrone-benzylidene-acetophenone*, $C_6H_5.CH(C_{14}H_9O)CH_2.CO.C_6H_5$.

By condensation of anthrone with benzylidene-acetophenone, Meerwein (10) obtained anthrone-benzylidene-acetophenone, according to the following reaction:



The same compound was obtained by the writers by treating β -anthronyl- β -phenyl-propionyl chloride dissolved in benzene with aluminium chloride.

Preparation.— β -Anthronyl- β -phenyl-propionyl chloride (20 gm.) was dissolved in 200 cc. of anhydrous benzene in a three-necked flask fitted with a mercury seal stirrer and reflux condenser and 16 gm. of aluminium chloride was added. A reddish color appeared. The mixture was heated on a water bath at 20° C. for three and a half hours. The temperature was raised till the benzene was boiling, when there was a rapid evolution of hydrochloric acid. After five hours' stirring, the mixture was cooled and poured on broken ice; a small quantity of hydrochloric acid was added and benzene distilled off with steam.

The reaction product was a reddish mass which solidified on cooling and was easily pulverized. It was a mixture of anthrone-benzylidene-acetophenone and two tautomeric substances which will be described below.

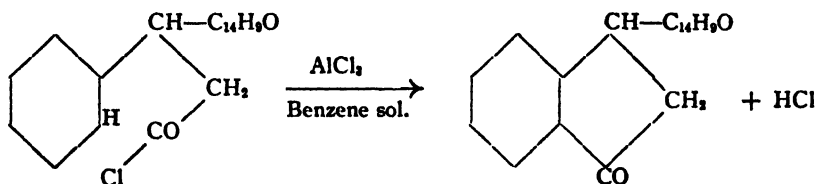
In order to separate anthrone-benzylidene-acetophenone, the raw product was dissolved in the least possible quantity of boiling glacial acetic acid. By cooling, about 8 gm. of a mixture of the two tautomeric substances separated out. They were filtered off and the filtrate containing anthrone-benzylidene-acetophenone was evaporated to dryness. The residue was dissolved in ether and the ethereal solution washed with concentrated ammonia (violet color) and with water. The anthrone-benzylidene-acetophenone remaining behind was recrystallized. It had a light pink color. After recrystallization from alcohol it was colorless; m.p. 115-116° C. Analysis:—Calcd. for $C_{20}H_{22}O_2$: C, 86.56; H, 5.47%. Found: C, 86.61, 86.60; H, 5.51, 5.53%.

Part III

γ-ANTHRONYL-*α*-HYDRINDONE AND ITS DERIVATIVES

In this section will be discussed:—(a) the methods by which *γ*-anthronyl-*α*-hydrindone was prepared and isolated in two tautomeric forms; (b) some derivatives of this ketone; and (c) the oxidation of *γ*-anthronyl-*α*-hydrindone.

I. *γ*-Anthronyl-*α*-hydrindone. The synthesis of this ketone was effected by the action of anhydrous aluminium chloride on *β*-anthronyl-*β*-phenyl-propionyl chloride dissolved in benzene.

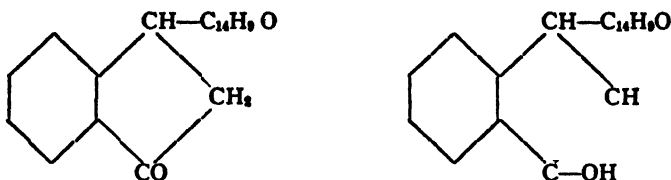


Moreover the benzene reacts with the acid chloride forming fairly large quantities of anthrone-benzylidene-acetophenone. The isolation of this compound was not very difficult, for it is much more soluble in acetic acid than are the other reaction products.

On the other hand, the purification of *γ*-anthronyl-*α*-hydrindone is more difficult, for the product separated from anthrone-benzylidene-acetophenone was a mixture of two compounds. It was necessary to recrystallize many times from methyl alcohol to separate them. One crystallizes in diamond-shaped crystals melting at 161-162° C., the other in monoclinic plates melting at 187-188° C.

Theoretically the two compounds obtained could be:—(a) two isomeric ketones containing different rings; or (b) the enolic form and the ketonic form of the same ketone.

It has been possible to show, as will be seen below, that the two substances are really the two tautomeric varieties of the same ketone:



Preparation.— β -Anthranyl- β -phenyl-propionyl chloride (20 gm.) was dissolved in 200 cc. of anhydrous benzene in a three-necked flask fitted with a mercury seal stirrer and reflux condenser. On the addition of 16 gm. of aluminium chloride, a reddish color appeared. The flask was placed in a water bath at 20°C . and the mixture stirred for three and a half hours. The temperature was then raised till the benzene was boiling, when there was a rapid evolution of hydrochloric acid. After stirring for five hours at that temperature the mixture was cooled and poured on broken ice; a small quantity of hydrochloric acid was added and the benzene was distilled off with steam. The reaction product was a reddish mass which solidified on cooling. This product was a mixture of anthrone-benzylidene-acetophenone and two tautomeric substances which will be described below.

In order to separate anthrone-benzylidene-acetophenone, the raw product was dissolved in the least possible quantity of boiling glacial acetic acid. On cooling, about 8 gm. of a solid mixture melting between 140 and 183°C . separated out. It was filtered off, washed with concentrated ammonia, dried and recrystallized once more from acetic acid. By fractional recrystallization from methyl alcohol two products were obtained:

(A) A colorless solid melting at 161 - 162°C . and separating from alcohol in diamond-shaped crystals.

(B) A colorless solid melting at 187 - 188°C . separating in monoclinic crystals.

The results of analyses and the determination of the molecular weights of these two compounds show that they are isomers. Analysis:—(A) Calcd. for $\text{C}_{22}\text{H}_{16}\text{O}_2$: C, 85.15; H, 4.96%. Found: C, 85.05, 85.18; H, 5.11, 4.98%. Mol. wt.:—Calcd. 324. Found: 311, 336, 318 (Landsberger). (B) Found: C, 85.09; H, 5.08%. Mol. wt. 315, 335.

γ -Anthranyl- α -hydrindone is formed by the method just described, but the yield of pure product is small because the solvent (benzene) reacts to form anthrone-benzylidene-acetophenone.

The yield was increased by the use of carbon disulphide as solvent and the preparation facilitated, for the double aluminium salt formed, which is soluble in benzene, is insoluble in carbon disulphide and could be filtered off. The ketone obtained by the decomposition of the double aluminium salt by water was almost pure and after one crystallization in toluene was composed almost exclusively of the variety melting at 162°C .

β-Anthranyl-*β*-phenyl-propionyl chloride (57 gm.) was dissolved in 285 cc. of anhydrous carbon disulphide, 59 gm. of aluminium chloride added and the mixture boiled under a reflux condenser for five hours, after which the evolution of hydrochloric acid had practically ceased and the double aluminium salt readily separated. It was filtered off and decomposed with water. Hydrochloric acid was added and steam passed through the mixture for a few minutes. The solid product was filtered off, washed with water, treated with a hot saturated solution of sodium bicarbonate and filtered off again. *β*-Anthranyl-*β*-phenyl-propionic acid (6.7 gm.) was obtained by acidifying the filtrate with sulphuric acid.

The dried residue weighed 46.2 gm. and melted at 140-145° C. By recrystallization from toluene, 33.5 gm. was obtained. Yield, 75%; m.p. 159-160° C. After many crystallizations from alcohol the product melted at 161-162° C.

γ-Anthranyl-*α*-hydrindone is slightly soluble in ether and carbon disulphide, fairly soluble in methyl and ethyl alcohol and very soluble in benzene and toluene. From alcohol and toluene, it separates in diamond-shaped crystals, whereas from carbon disulphide and benzene it separates in needles containing solvent of crystallization. Cold concentrated sulphuric acid dissolves it to form a greenish-yellow solution. The solution was poured into water and the unaltered ketone precipitated. When treated with concentrated alkalis the ketone develops a very intense reddish brown color and partly dissolves. It appears to be more stable towards acids than alkalis.

γ-Anthranyl-*α*-hydrindone is also formed by dehydration of *β*-anthranyl-*β*-phenyl-propionic acid. The temperature at which the experiment must be performed to obtain only one of the two tautomeric forms of the ketone has been determined. *β*-Anthranyl-*β*-phenyl-propionic acid (4 gm.) was dissolved in 100 cc. of concentrated sulphuric acid and the solution heated at 103-104° C., when a very sharp change in color was produced. The mixture, up to this point yellow, rapidly became violet colored. This change is probably due to the fact that, besides *γ*-anthranyl-*α*-hydrindone, Bz-1-oxy-Bz-3-phenyl-benzanthrone is formed. This compound develops with sulphuric acid a very intense reddish color (5).

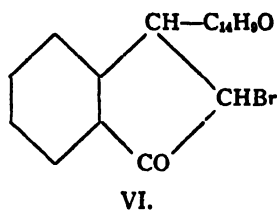
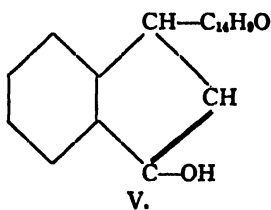
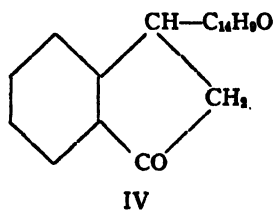
The solution was heated at 110° C. for three minutes and then poured on ice. The ketone precipitated and was filtered off. It was boiled with dilute ammonia (very intense violet color). The ketone, insoluble in ammonia, was filtered off and dried at 100° C.; yield, about 35%. The ketone crystallizes from toluene almost without color and melts at 161-162° C. It was proved identical with *γ*-anthranyl-*α*-hydrindone by a mixed melting point determination.

When the raw *γ*-anthranyl-*α*-hydrindone obtained by any one of the three methods just described was being purified, it was noticed that the alkaline solution used to purify the ketone developed a very intense violet color, which was particularly intense in the case of the preparation of *γ*-anthranyl-*α*-hydrindone with sulphuric acid. It may be due to the presence of traces

of phenyl-benzanthrone since Bz-1-oxy-Bz-3-phenyl-1-9-benzanthrone treated with alkalis becomes violet colored (5). This has not been proved.

II. γ -Anthronyl- α -hydrindone Derivatives

1. γ -Anthronyl- β -brom- α -hydrindone. As previously stated, when β -anthronyl- β -phenyl-propionyl chloride is treated with aluminium chloride two different compounds are formed, one melting at 162°C ., the other at 188°C . The writers ascribe to them the formulas IV and V.

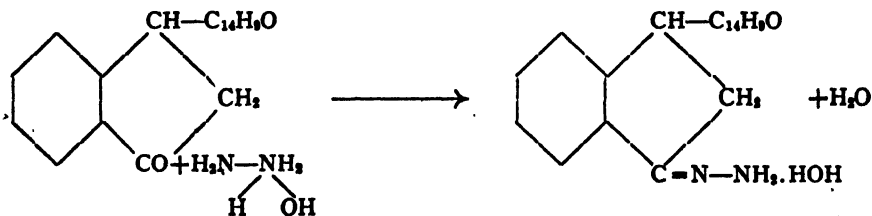


The writers believe that the two products are tautomers since each gives rise to the same monobromide VI.

Preparation of the monobromide from the product melting at 162°C .—Six grams of the compound melting at 162°C . and 350-400 cc. of ether were treated with bromine (2.8 gm.) and the mixture boiled. After a few minutes, the solution was clear and rapidly became colorless, when crystals of the monobromide were deposited on the wall of the flask. The contents was kept boiling for five hours. After cooling, the crystalline product was filtered off, washed many times with ether and dried in the air. Yield, 6.4 gm.; m.p. 166 - 167°C . Recrystallized from alcohol, the compound melts at 169 - 171°C . It is colorless and easily soluble in benzene and toluene; and slightly soluble in carbon disulphide and ether. It separates from alcohol in prisms. Analysis:—Calcd. for $\text{C}_{23}\text{H}_{18}\text{O}_2\text{Br}$: Br, 19.82%. Found: Br, 19.60%.

The monobromide from the compound melting at 188°C .—This monobromide was prepared in a manner similar to the one just described. The yield of bromide from 3 gm. of material was 3.2 gm.; m.p. 169 - 171°C . It is slightly soluble in hot carbon disulphide and hot ether. It is very soluble in hot ethyl or methyl alcohol. Analysis:—Calcd. for $\text{C}_{23}\text{H}_{18}\text{O}_2\text{Br}$: Br, 19.82%. Found: Br, 19.78%. Both bromides were proved identical by a mixed melting point determination.

2. γ -Anthronyl- α -hydrindone hydrazone.—The hydrazone was prepared by heating an alcoholic solution of the ketone with hydrazine hydrate. The following reaction takes place:



A hydrate of the hydrazone is obtained.

Twenty grams of the ketone melting at 162° C. was dissolved in the minimum quantity of hot alcohol. Hydrazine hydrate (7.1 gm., 42% in water) was added and the mixture boiled for five hours. The hydrazone, less soluble than the ketone, separated on the walls of the flask. The contents of the flask was cooled at 0° C., the hydrazone filtered off and pressed on a porous porcelain plate. Yield, 17.9 gm. (81.7%).

The hydrazone crystallizes from alcohol in colorless prisms melting with decomposition at 176° C. Analysis:—Calcd. for $C_{23}H_{18}ON_2 \cdot H_2O$: N, 7.87%. Found, 7.85%.

3. *γ*-Anthronyl-*α*-hydrindone phenylhydrazone.—Three grams of the ketone was dissolved in 25-30 cc. of glacial acetic acid, the solution cooled to room temperature and treated with 3 cc. of phenylhydrazine. After standing for ten hours the phenylhydrazone was deposited. It was filtered off and washed many times with alcohol and ether. Yield, 3.9 gm. It was purified by recrystallization from toluene from which it separated in yellow needles; m.p., 224-225° C. It is soluble in toluene and benzene, slightly soluble in alcohol and ether, and insoluble in petroleum ether. The phenylhydrazone crystallized from toluene contains one molecule of this solvent. Analysis:—Calcd. for $C_{29}H_{22}ON_2 \cdot C_6H_6 \cdot CH_3$: N, 5.53%. Found: N, 5.42, 5.52%.

4. *γ*-Anthronyl-*α*-hydrindone semicarbazone.—Of the *γ*-anthronyl-*α*-hydrindone derivatives the semicarbazone is most readily obtained.

Four grams of the ketone was dissolved in the minimum quantity of hot 95% alcohol. A mixture of 1.42 gm. of semicarbazide hydrochloride and 1.78 gm. of fused sodium acetate, in aqueous solution, was added to the boiling mixture. The solution was boiled under a reflux condenser for six hours. The slightly soluble semicarbazone precipitated and after cooling was filtered off, washed with water and finally with alcohol and ether. Yield, 3.5 gm. The semicarbazone is slightly soluble in most organic solvents. Recrystallized from alcohol it is obtained pure and melts at 243° C. Analysis:—Calcd. for $C_{24}H_{18}O_2N_3$: N, 11.02%. Found: 10.95%.

5. *γ*-Anthronyl-*α*-hydrindone oxime.—Five grams of the ketone was dissolved in the minimum quantity of alcohol. A mixture of 2.3 gm. of hydroxylamine hydrochloride and 2.7 gm. of fused sodium acetate, in aqueous solution, was added to the boiling alcoholic solution which was kept boiling for 12 hr. After cooling, the solution was filtered and poured into one litre of water. Sulphuric acid was added and the precipitated oxime filtered off, washed with water, and dried. Yield, 4 gm.; m.p. 178-180° C. The oxime was recrystallized from toluene. It was almost colorless, and melted at 181-184° C. The oxime is very soluble in alcohol and ether, less soluble in benzene and toluene. When crystallized from toluene it retains one molecule of solvent, as shown by the results of the analysis. Analysis:—Calcd. for $C_{23}H_{17}O_2N$: N, 3.24%. Found: N, 3.26, 3.25%.

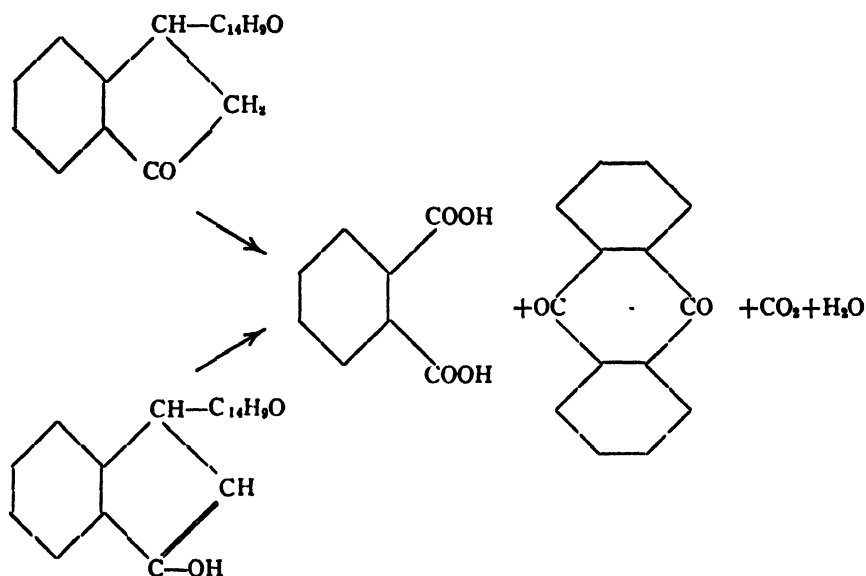
III. Oxidation Products of γ -Anthronyl- α -hydrindone

The analyses and the determination of the molecular weights of the two tautomeric forms of the γ -anthronyl- α -hydrindone show that these compounds have the formula $C_{22}H_{18}O_2$. They may then possess the structure of one of the two theoretically possible ketones (I, II) or of their tautomers.

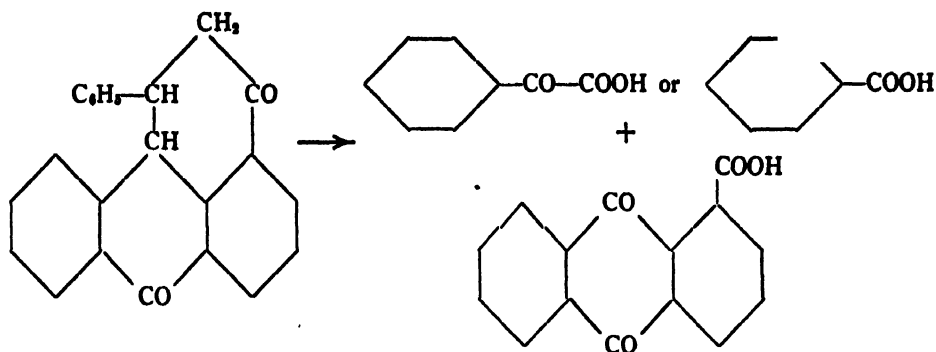
By oxidizing the compounds obtained it has been proved that they are α -hydrindone derivatives. Chromic acid was first used and then nitric acid as oxidizing agent. By using Liebermann and Roka's method (9) and Halley and Marvel's method (3), with chromic acid, anthraquinone was obtained almost exclusively.

On the other hand with nitric acid (sp. gr., 1.2) according to König's method (8) a large quantity of phthalic acid was obtained, besides anthraquinone.

The formation of phthalic acid proves that the tautomers are α -hydrindone derivatives. Oxidation takes place as shown by the following diagram:



The phthalic acid could be formed by oxidation of anthraquinone, but it has been shown that the phthalic acid obtained does not arise from anthraquinone. In fact almost the calculated quantity of anthraquinone and phthalic acid was obtained. This acid then does not come from anthraquinone but from the hydrindone ring. The tautomers produced do not contain the benzanthrone ring; if they did, oxidation would not give rise to anthraquinone but rather to acids, according to the following scheme:



The constitution of the products prepared is then well established.

Oxidation with nitric acid.—The ketone (3.8 gm.) was mixed with 100 cc. of nitric acid (sp. gr., 1.2) and heated to boiling. The ketone was gradually oxidized, a rapid evolution of nitrous vapors taking place. After two hours, the reaction was complete; the mixture was allowed to cool and the yellow insoluble solid filtered off. Dried at 80°C . it weighed 2.4 gm. It was purified by crystallization in acetic acid. There was thus obtained 1.3 gm. of very fine yellow needles melting at 278°C . This product was easily identified as anthraquinone by a mixed melting point determination.

The filtrate was evaporated to dryness on the water bath. About 1.3 gm. of a resinous product remained. It was carefully sublimed and long colorless needles of phthalic anhydride melting at 128°C . were obtained. These crystals were dissolved in boiling water, the aqueous solution filtered and evaporated to dryness. A crystalline colorless product melting at 187°C . was thus obtained. It was identified as phthalic acid by a mixed melting point determination.

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ERRATA

Page 491, Reference 2, for "food-rotting fungi" read "foot-rotting fungi."

Page 578, line 2, under "Materials," for "100°C" read "1000°C."

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